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PART I

A PARTIAL STRUCTURAL FORMULA FOR FLAVENSOMYCIN

PART II

STUDIES OF NUCLEAR MAGNETIC NONEQUIVALENCE OF METHYLENE PROTONS

A THESIS

Presented to

The Faculty of the Graduate Division

by

Howard Mark Deutsch

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

Georgia Institute of Technology

April, 1967

PART I

A PARTIAL STRUCTURAL FORMULA FOR FLAVENSOMYCIN

PART II

STUDIES OF NUCLEAR MAGNETIC NONEQUIVALENCE

OF METHYLENE PROTONS

Approved:

Chair

Date approved by Chairman: 4/19/67

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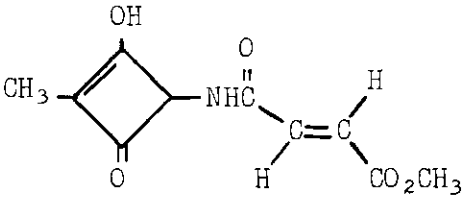
GLOSSARY OF ABBREVIATIONS

AIP	Argon inlet pressure (GLC).
C_n	A carboxylic acid containing n carbon atoms, not including substituent methoxyl groups.
CT	Column temperature (GLC).
cps	Cycles per second.
DC	Dicarboxylate (methyl ester).
DSS	Sodium 2,2-dimethyl-2-silapentane-5-sulfonate, n.m.r. standard.
FR	Flow rate (GLC).
GLC	Gas-liquid chromatography.
Mc	Megacycles (per second).
MC	Monocarboxylate (methyl ester).
MS	Mass spectrometry.
MW	Molecular weight.
ppm	Parts per million (n.m.r.).
QF-1	Liquid phase for GLC.
RT	Retention time (GLC).
SE-30	Liquid phase for GLC.
TC	Tricarboxylate (methyl ester).
TLC	Thin-layer chromatography.
TMS	Tetramethylsilane, n.m.r. standard.
w,m,s,v	Weak, medium, strong, and very; used to designate intensities of spots in TLC.

SUMMARY

PART I

Flavensomycin is a potent, toxic antibiotic that was first isolated in 1957. It was shown to be very active against certain fungi and insects, but had almost no antibacterial activity. Flavensomycin was also very toxic toward higher animals; this discouraged possible application to human subjects. The toxicity toward mice was about the same as sodium cyanide. Flavensomycin was reported to be a monobasic acid and had pK_a 5.8, equivalent weight 740. Various analytical data showed that it contained carbon, hydrogen, and nitrogen, but no sulfur or halogens. The formula $C_{38}H_{58}N_2O_{10}$ (molecular weight, 702.9) was suggested. The spectral properties of flavensomycin that have been reported (infrared and ultraviolet) indicated that it contained conjugated, unsaturated groups. Methanolysis of flavensomycin in the presence of an acidic ion-exchange resin was reported to yield a crystalline compound, named flavensomycinic acid. The chemical and spectroscopic properties of flavensomycinic acid lead to a proposal of structure I. However, a rational synthesis of structure I revealed that it was not identical to flavensomycinic



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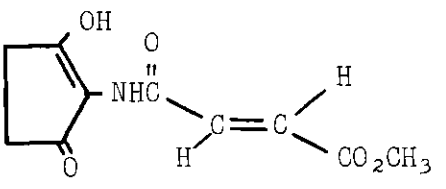


II

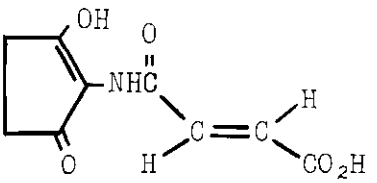
acid. The only other reasonable structure for this acid appeared to be II. Structure II was synthesized in a straightforward manner and was reported to be identical in physical and spectroscopic properties to the natural product.

The purpose of this research was to establish as many of structure features as possible of the antibiotic, flavensomycin. Base on part of our results, it is possible that flavensomycin is a macrocyclic lactone (macrolide). Generally, the macrolide antibiotics are both antifungal and antibacterial and do not have significant ultraviolet chromophores. However, the polyene macrolides are only antifungal and have very characteristic ultraviolet absorption. Flavensomycin is not, however, a typical polyene macrolide based on its ultraviolet absorption. In addition, many of the macrolide antibiotics contain unusual sugar components; there is no evidence that flavensomycin has a sugar component. On the other hand, methanolysis of flavensomycin produces the unusual compound flavensomycinic acid (II). It thus appears that flavensomycin is a new type of antibiotic, and knowledge of its structure would add greatly to data on structure-activity-toxicity relationships, particularly in the macrolide-polyene group of antibiotics.

The material used in this investigation was shown to be flavensomycin by the identity of its physical and spectroscopic properties to those reported in the literature. The molecular weight of flavensomycin was determined by vapor pressure osmometry and mass spectrometry (MS); these values, along with the analytical data that have been obtained, established the formula $C_{47}H_{69}NO_{14}$ (molecular weight, 872.07). In addition, flavensomycin contained approximately ten C-CH_3 groups, three -OCH_3

groups, and eight to ten active hydrogens.

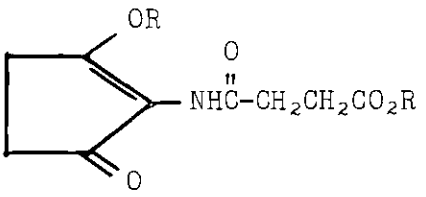
Flavensomycin was very unstable, and was degraded quite readily in a number of common organic solvents. It was also degraded rapidly when chromatographed on silicic acid; one of the products from the degradation was shown to be flavensomycinoic acid (III). Several relatively mild re-



III

actions on flavensomycin, such as reduction with sodium borohydride, degradation with boiling chloroform, and methanolysis (the products besides flavensomycinic acid) were shown to yield complex mixtures of products.

Flavensomycin was readily reduced under catalytic hydrogenation conditions. About seven and four moles/mole of hydrogen were absorbed when acetic acid and ethanol, respectively, were used as solvents. The product from the acetic acid reduction could be resolved into two main components by silicic acid chromatography. One of these components was shown to be dihydroflavensomycinoic acid (IV). The mass spectrum of dimethyl dihydroflavensomycinoate (V) was fully consistent with the proposed structure. The other main component from the reduction in acetic acid was a colorless oil, and was named the perhydro fragment. This material was probably a mixture of several (closely related) components. An infrared spectrum of this material showed, among others, a peak at 5.74 μ, which is characteristic of lactones (and esters).

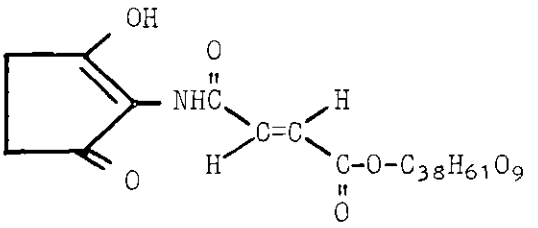


IV R = H; V R = CH_3

Oxidation with concentrated nitric acid is a common technique used in the structure determination of antibiotics. Generally, dicarboxylic acids are the main products. The nitric acid oxidations of flavensomycin and perhydroflavensomycin (from reduction in acetic acid) were shown to yield as the volatile products, acetic, propionic, and isobutyric acids, when analyzed as their p-phenylphenacyl esters by gas-liquid chromatography (GLC). The nonvolatile products, when converted to methyl esters and analyzed by GLC were shown to be complex mixtures of ca. 40 components. Often it is possible to obtain much more information about complex mixtures by using the combined techniques of mass spectrometry - gas-liquid chromatography (MS-GLC). To this end, a number of known dicarboxylic acids were converted to their methyl esters, and their mass spectra were determined by MS-GLC. The methyl esters from the nitric acid oxidation of flavensomycin were also analyzed by this technique and the following components were identified (as methyl esters): oxalate, pimelate, suberate, azelate, sebacate, undecandioate, dodecanedioate, and stearate. A number of methoxy esters (structures not determined) were also detected. From the nitric acid oxidation of perhydroflavensomycin the following components were identified: succinate, 2-methylsuccinate, glutarate, 2-methylglutarate, 2,4-dimethylglutarate (both isomers),

stearate, and tentatively either CH_3O_2C-$C(CH_3)(CH_2CH_3)$-CH_2-$C(CH_3)(CH_2CH_3)$-CO_2CH_3 or CH_3O_2C-$C(CH_3)(CH_2CH_3)$-$C(CH_3)_2$-$CH_2CH(CH_3)CO_2CH_3$. A number of methoxy esters were also detected.

The $\underline{p}K_a$ value of flavensomycin suggested that the attachment of the flavensomycinoic acid portion to the remaining part of the molecule was through the carboxyl group. This was also in accord with the n.m.r. data. Thus, the following part structure is proposed for flavensomycin.



The $C_{38}H_{61}O_9$ fragment must satisfy the following conditions: contain eight double bonds and/or rings; have six double bonds and/or rings that are easily reduced (platinum in acetic acid), and therefoire two double bonds and/or rings that are not easily reduced; contain about three $-OCH_3$ groups and about ten $C-CH_3$ groups; have about six to eight active hydrogens; contain one group that is capable of consuming base; and satisfy in part the structures of the acids produced by nitric acid oxidation. When flavensomycin is reduced to perhydroflavensomycin, the presumed fragment $C_{38}H_{73}O_9$ must in addition satisfy in part the structures of the acids produced by nitric acid oxidation.

PART II

Nuclear magnetic resonance spectroscopy (n.m.r.) has rapidly grown into one of the most widely practiced techniques that the chemist may use.

One area of n.m.r. research has dealt with the determination of the nature of the chemical shift difference that is sometimes observed between atoms or groups that are otherwise equivalent. As early as 1956 it was recognized that the geminal atoms or groups (R) in compounds of the type ACR_2-CXYZ (Z can equal $-CR_2A$) were often magnetically nonequivalent. Basically two theories have been advanced to explain this phenomenon; the "conformer preference theory," and the "intrinsic asymmetry theory." The object of this research was to synthesize compounds of the above mentioned type (with Z equal to $-CR_2A$); by studying how the n.m.r. parameters of such a system vary, by changing both the substituent groups (A,X,Y) and the solvents used for determination, it was hoped that some insight could be gained into this problem.

A series of compounds was synthesized with substituent groups such as $-CO_2H$, $-CO_2CH_3$, $-CONH_2$, -Cl, -Br, and -CN. The n.m.r. spectra of these compounds were determined in deuteriochloroform, carbon tetrachloride, benzene, acetone-d_6, pyridine, and deuterium oxide, depending on the solubility characteristics of the given compound. These spectra were analyzed by a trial and error method, using a program written for the Burroughs B-5500 computer.

Large changes with solvent were observed in the chemical shift values and the differences in chemical shifts for protons within a given molecule. These changes did not seem to be related to such properties of the solvent as dielectric constant or polarity. Benzene solvent did not exert a specific shielding effect, as is usually observed. The lack of an observable solvent dependency of the vicinal coupling constants was interpreted in terms of small or negligible changes in

conformer population with solvent. It thus appeared that chemical shift data were not a reliable measure of conformer population; this is in agreement with the work of others.

The equation developed by Snyder that relates conformer population with the vicinal coupling constants was used to calculate values of K, the ratio of conformer populations. In particular, the values obtained for 1,2,3-tribromopropane, and possibly tricarballylic acid did not seem reasonable. No relationship between K and the chemical shift differences between geminal protons was observed, perhaps indicating that the populations of the various conformers were not important in determining the extent of magnetic nonequivalence of these protons.

In general, these data seemed to offer more support for the "intrinsic asymmetry theory," but did not exclude the possibility that the "conformational preference theory" was the the most important effect.

CHAPTER I

INTRODUCTION

Flavensomycin

The potent, toxic antibiotic flavensomycin was first isolated from a culture broth of Streptomyces cavourensis in 1957 (1). Flavensomycin was shown to be active against a wide variety of fungi. In particular, it was active against yeasts such as Saccharomyces (0.06-0.25 μg./ml.) and against molds such as Penicillium (0.03-3.0 μg./ml.) (2). However, it was inactive against bacteria at levels of less than 100 μg./ml. (1). Flavensomycin also showed good insecticidal activity, including insects such as Musca domestica and Locusta migratoria. Its activity against the former insect was shown to be ten times greater than DDT (1). Flavensomycin was not toxic toward higher plants and was shown to protect corn plants from the fungus Helminthosporium turcicum. It also inhibited infection of bean leaves by tobacco mosaic virus but did not inactivate the tobacco mosaic virus per se. (3).

Flavensomycin is very toxic toward higher animals; this discouraged possible use with human subjects. The LD_{50} (mice) was shown to be 1 mg./kg. intraperitoneally, 2 mg./kg. subcutaneously, and 19-25 mg./kg. orally (1).

Generally, flavensomycin was isolated by the following procedure (1): the culture broth was extracted with benzene, and petroleum ether was added to the benzene extract; the crude precipitate was washed with petroleum ether and dissolved in acetone; the insoluble portion was discarded; petroleum ether was added to the acetone solution, and the precipitate

was collected, dissolved in benzene, and chromatographed on alumina; elution with ethyl acetate-ethanol gave colored inactive fractions; elution with methanol yielded the antibiotic, which was rechromatographed on alumina and eluted with 5% methanol in benzene. Flavensomycin isolated by this procedure could be crystallized from benzene-petroleum ether as yellow, tabular crystals that showed m.p. 152 $\pm$ 2° (1), or m.p. 130-131° (with evolution of a gas, resolidification, and remelting (dec.) at ca. 200°) (4).

Flavensomycin gave positive Molish, Fehling's, and Ehrlich tests but negative Tollen's, Seliwanoff, Millon, Liebermann, Sakaguchi, and biuret tests (2). Potentiometric titration showed that flavensomycin was a monobasic acid, and had pK_a 5.8, equivalent weight 740 in 3:1 ethanol-water (4) or pK_a 6.08, equivalent weight 815 in 1:1 ethanol-water (5). Various analytical data showed that it contained carbon, hydrogen, and nitrogen, but no sulfur or halogens. The formula $C_{38}H_{58}N_2O_{10}$ (molecular weight, 702.9) has been suggested for flavensomycin (4).* The same authors report that flavensomycin contained two O-CH_3 groups (found: O-CH_3, 9.18%) and three C-CH_3 groups (found: C-CH_3, 10.30%).

Other physical properties of flavensomycin which have been reported are: ultraviolet absorption, λ_{max}^{MeOH} 251 mμ** (1), λ_{max}*** 249-252, 282

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* This formula was based on analytical data which reported 3.85% nitrogen. This value is approximately twice that reported in other work (5,6,7), and thus appears to be in error.

** No extinction coefficient was quoted.

*** The solvent used was not quoted.

(sh.), and 337.5 mμ (sh.) ($E^{1\%}_{1\ cm}$ 632.2, 276.0, and 67.6) (5); infrared absorption, λ_{max}* 2.90, 3.11, 3.26, 3.45, 5.86, 5.94, 6.17, 6.51, 6.92, 8.02, 9.05, 10.04, 10.32, 10.91, 13.02, and 13.13 μ (4,6) or λ_{max}* 2.92, 3.08, 3.25, 5.80, 5.94, 6.18, 6.44, 8.04, 8.55, 9.09, 10.38, 10.59, 10.93, and 13.17 μ (5).

Flavensomycin was reduced in methanol with hydrogen and 10% palladium on carbon; about four moles of hydrogen per mole of flavensomycin were consumed (4). When flavensomycin was warmed with a 15% potassium hydroxide solution, acetaldehyde, isobutryaldehyde (identified as the 2,4-dinitrophenylhydrazone derivatives), and ammonia (identified as a p-hydroxyazobenzene-p'-sulfonate salt) were produced (4).

Methanolysis of flavensomycin, in the presence of Dowex-50-X8, was shown to yield a crystalline compound (8,9) that was named flavensomycinic acid (I). Analytical results indicated that its formula was $C_{10}H_{11}NO_5$ (molecular weight 225.2). Compound I was a yellow solid, had m.p. 232-233°, was optically inactive, and contained one C-CH_3 group (Kuhn-Roth)** and one O-CH_3 group (Zeisel). Potentiometric titration showed that it had pK_a 5.64 (equivalent weight 221) in 3:1 ethanol-water. Compound I showed infrared absorption (nujol mull) at λ_{max} 3.13, 5.80, 6.20, 6.40, 7.72, 8.53, 10.07, 13.10, and 15.00 μ, ultraviolet absorption at λ^{MeOH}_{max} 259 and 330 mμ (ε 24,000 and 2950***). In addition, it gave positive ferric chloride and hydroxamic ester tests, but negative ninhydrin,

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* The method of determination was not stated.

** Acetic acid was identified by paper chromatography.

*** Reference (8) quoted 8300 for this value, and probably is in error.

Ehrlich and triphenyltetrazolium tests.

Ozonolysis of the sodium salt of I in water yielded methyl glyoxylate (identified as the 2,4-dinitrophenylhydrazone derivative). Oxidation of I with chromium trioxide in acetic acid yielded methyl fumaramate, the structure of which was proved by comparison with a synthetic sample. Vigorous acidic or basic hydrolysis of I produced fumaric acid.

Mild alkaline hydrolysis of I produced flavensomycinoic acid (II), $C_9H_9NO_5$ (molecular weight, 211.2) which was a yellow crystalline solid, m.p. 249-251°. Potentiometric titration showed that it had $\underline{p}K_a$ 3.5 and 4.7 in water. Compound II could be reconverted to I by treatment with methanol in the presence of Dowex-50-X8. Catalytic reduction of II produced dihydroflavensomycinoic acid (III) which was obtained only as an aqueous solution (the free acid was not isolated). However, potentiometric titration indicated that it had $\underline{p}K_a$ 2.8 and 3.6 in aqueous solution.

Catalytic hydrogenation of I produced dihydroflavensomycinic acid (IV), $C_{10}H_{13}NO_5$ (molecular weight, 227.2). Compound IV was a white, crystalline solid and had m.p. 135°; it showed infrared absorption (nujol mull) at λ_{max} 3.80 (broad) and 5.76 μ, ultraviolet absorption (methanol) at $\lambda_{max}^{0.01\ \underline{N}\ NaOH}$ 259 mμ (ε 23,100), $\lambda_{max}^{0.01\ \underline{N}\ HCl}$ 252 and 259 mμ (sh.) (ε 8900 and 8300), and n.m.r. absorption (deuteriochloroform) at ca. 7.36 (8H, two broad singlets), 6.28 (3H, singlet), 1.66 (1H, broad singlet), and -2.96 τ (1H, singlet). In addition, both the hydroxamic ester and ferric chloride tests were positive.

Vigorous acidic or basic hydrolysis of IV yielded succinic acid (identified as the bis-p-bromophenacyl ester derivative). Oxidation of

IV with sodium periodate in neutral solution at 0° yielded oxalic and succinamic acids (identified by paper chromatography). By strong alkaline hydrolysis of IV a mixture of products was obtained; these products reduced triphenyltetrazolium chloride. When the above mixture was oxidized with basic hydrogen peroxide, propionic acid could be detected by paper chromatography. Treatment of IV with an excess of bromine in carbon tetrachloride gave a monobromo derivative, $C_{10}H_{12}NO_5Br$, m.p. 115-116°. This derivative reduced triphenyltetrazolium chloride in the cold.

Treatment of IV with an excess of diazomethane yielded dimethyl dihydroflavensomycinoate (V)*, $C_{11}H_{15}NO_5$ (molecular weight 241). Compound V was a white crystalline solid, m.p. 117-118°; the molecular weight, as determined by the Rast method, was 248. It showed infrared absorption (nujol mull) at λ_{max} 5.75, 5.91, and 7.95 μ, ultraviolet absorption at λ_{max}^{MeOH} 249 mμ**, and n.m.r. absorption (deuteriochloroform) at 7.37 (3H, singlet), ca. 7.37 (5H, multiplet), 6.35 (3H, singlet), 5.97 (3H, singlet), and 2.33 τ (1H, broad singlet). In addition, V gave a negative ferric chloride test and was very readily hydrolyzed to IV (even by recrystallization from solvents which were not completely anhydrous).

The various chemical transformations, which are discussed above, are outlined in Chart I.

The authors concluded that part structure VI could be written for compound I. The fragment $C_5H_5O_2$ must contain three double bonds and/or

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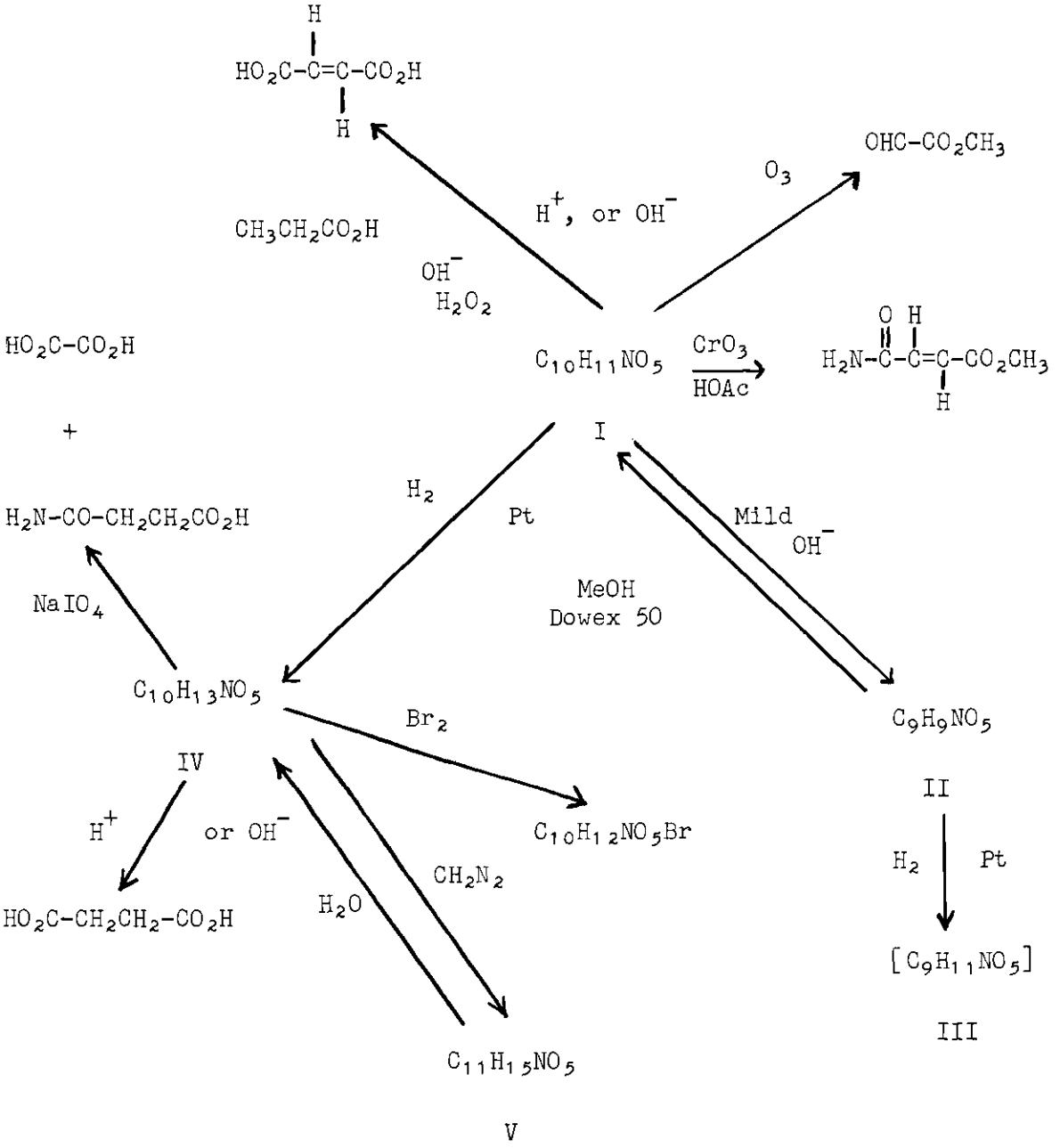
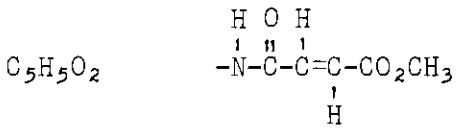
* The authors did not suggest a name for this compound, but the one given seems to follow logically.

** No extinction coefficient was quoted.

Chart 1. Chemical Transformations of Flavensomycinoic Acid and Related Compounds



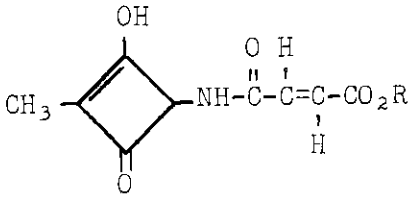


$C_5H_5O_2$

VI

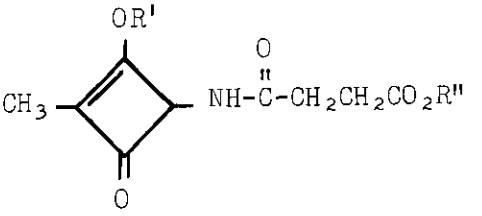
rings and must satisfy the following conditions: yield acetic acid with the Kuhn-Roth test; give propionic acid by vigorous basic hydrolysis followed by hydrogen peroxide oxidation; contain a strongly acidic proton; yield oxalic acid by neutral periodate oxidation; not be readily reduced by catalytic hydrogenation; contain a significant ultraviolet chromophore, satisfy the condition that $\Delta \lambda_{max}$ ($\Delta \lambda_{max} = \lambda_{max}^{anion} - \lambda_{max}^{acid}$) be approximately 10 mμ; and give a positive ferric chloride test. The above properties can only be readily satisfied by a 1,3-cyclobutanedione part structure. In particular, $\Delta \lambda_{max}$ has been measured for the series of 1,3-cyclodiones including cyclobutane, cyclopentane, and cyclohexanediones; $\Delta \lambda_{max}$ was ca. 10, 20, and 30 mμ, respectively. In addition, 1,1-diphenyl-2-ethoxy-3-methylcyclobut-2-ene-4-one and 1,3-diphenyl-2-ethoxy-cyclobutene-4-one are reported to have ultraviolet absorption at λ_{max}^{EtOH} 248 and 245 mμ, respectively (10). Thus, the structures shown below were proposed for compounds I-V.

Recently (11), Canonica, et al., have synthesized the compounds corresponding to the structures proposed for dihydroflavensomycinoic acid (III) and dimethyl dihydroflavensomycinoate (V). Cycloaddition of ethoxypropyne (VII) to succinimidoketene (VIII) yielded compound IX in 70% yield. Compound IX was a crystalline solid, m.p. 83°, and showed ultraviolet absorption at λ_{max}^{MeOH} 240 mμ (ε 11,500), infrared absorption (nujol mull) at λ_{max} 5.67, 5.87, and 6.16 μ, and n.m.r. absorption



I. $R = CH_3$

II. $R = H$

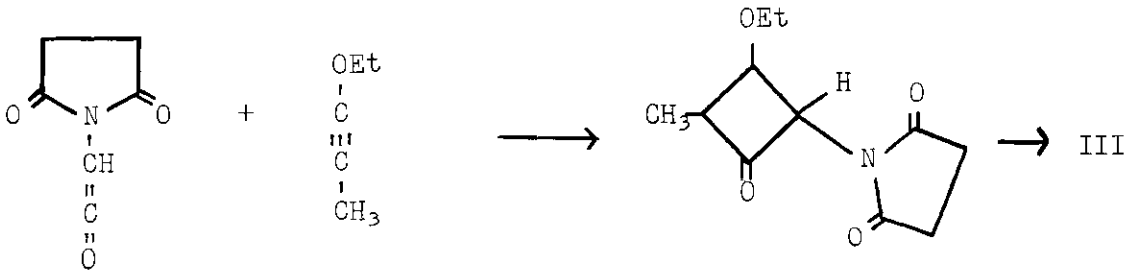


III. $R' = H$, $R'' = H$

IV. $R' = H$, $R'' = CH_3$

V. $R' = CH_3$, $R'' = CH_3$

(deuteriochloroform) at 8.59 (3H, triplet), 8.22 (3H, doublet J = 2.3 cps), 7.3 (4H, singlet), 5.62 (2H, quartet), and 4.6 τ (1H, quartet J = 2.3 cps).



VII VIII IX

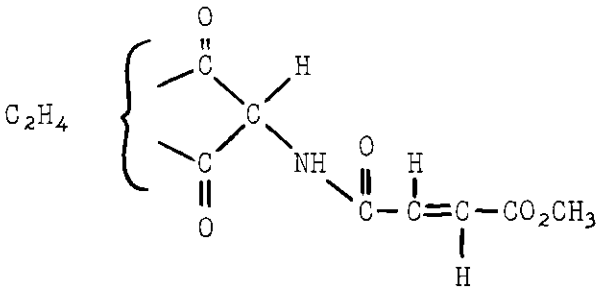
Mild alkaline hydrolysis of IX (0.2 N sodium hydroxide) yielded compound III. This compound showed m.p. 182°, ultraviolet absorption (methanol containing sodium hydroxide) at λ_{max} 251 (ε 17,800), infrared (nujol mull) absorption at λ_{max} 3.03, 3.28, 3.70-4.35, 5.76, 5.90, 6.04, and 6.48 μ, and n.m.r. (dimethyl sulfoxide-d_6) absorption at 8.54 (3H, doublet J = 2.3), 7.63 (4H, broad singlet), 4.84 (1H, doublet quartet $J_{H,NH} = 9$, $J_{H,CH_3} = 2.3$), 1.72 (1H, broad doublet J = 9), and -0.6 τ (2H,

singlet).

Compound III, upon treatment with an excess of diazomethane, yielded V. Compound V showed m.p. 130°, ultraviolet absorption at λ_{max}^{MeOH} 240 mμ (ε 12,600), infrared absorption (nujol mull) at λ_{max} 3.08, 3.30, 5.70, 5.80, 6.08, 6.19, and 6.49 μ, and n.m.r. (deuteriochloroform) absorption at 8.37 (3H, doublet J = 2.3), 7.41 (4H, multiplet), 6.36 (3H, singlet), 5.93 (3H, singlet), 4.58 (1H, double quartet $J_{H,NH}$ = 9, J_{H,CH_3} = 2.3), and 3.28 τ (1H, broad doublet J = 9). The authors did not comment on the unusually large long range coupling observed between the methyl group at C-3 and the hydrogen at C-1.

The observed properties of the synthetic compounds III and V were markedly different from the properties of the compounds isolated from methanolysis of flavensomycin and subsequent reactions. Thus, the authors concluded that the structures they had previously proposed were in error.

They pointed out that in their previous communication (8) the evidence had led them to part structure X, a 2-fumarylamino-1,3-cyclodione methyl ester, for flavensomycinic acid. The chemical and spectral

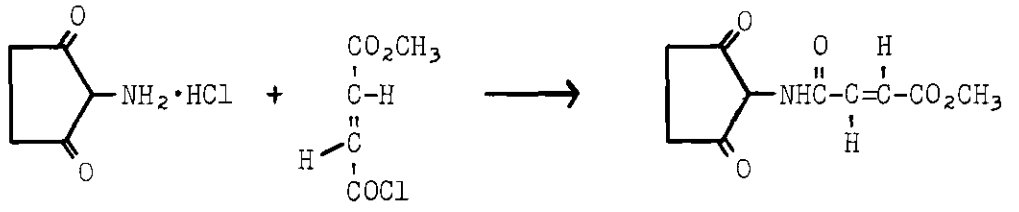


X

properties that were previously mentioned led them to propose that the

C_2H_4 fragment was CH_3-CH (they state that in particular the n.m.r. data were misinterpreted). In another study, Jommi et al. (12) found that the properties of other 2-acylamino-1,3-cyclodiones were very similar to those of the products derived from flavensomycin. If part structure X was correct, then the only other possible structure of the fragment C_2H_4 is $-CH_2CH_2-$, and the structure of flavensomycinic acid would be XIII.

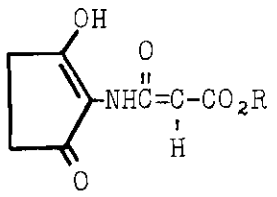
The reaction of 2-aminocyclopentane-1,3-dione hydrochloride (XI) with methyl fumaryl chloride (XIII) gave compound XIII (11). This syn-



XI XII XIII

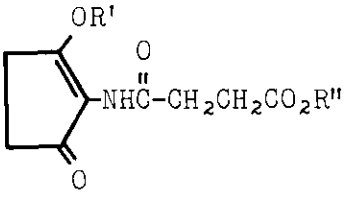
thetic sample was identical in all respects to flavensomycinic acid (mixed m.p., ultraviolet, infrared, and n.m.r. absorption). Catalytic hydrogenation of XIII produced dihydroflavensomycinic acid (XIV), which could be converted to dimethyl dihydroflavensomycinoate (XV) with an excess of diazomethane. Compounds XIV and XV were identical with the corresponding compounds from naturally derived flavensomycinic acid. Thus, dihydroflavensomycinoic acid should be assigned structure XVI and flavensomycinoic acid structure XVII.

Flavensomycin has also been shown to be a fermentation product of the microorganism Streptomyces griseus (3). The antibiotic humidin is also produced by this organism. Humidin was first isolated in 1958 from culture broths of Streptomyces humidus (13). It has strong antifungal



XIII. R = CH_3

XVII. R = H



XIV. R' = H, R" = CH_3

XV . R' = CH_3, R" = CH_3

XVI. R' = H, R" = H

activity (inhibits Hypochnus sasaki at levels of 0.5 μg/ml.) (14) and is also active against protozoa and certain fungi which cause plant diseases (13). It is also reported to be a white, optically active crystalline compound, m.p. 145-146° (from ethanol). The molecular weight was determined to be 550 ± 50 (Barger method) or 823 ± 10 (X-ray). The empirical formula $(C_{12}H_{20}O_4)_n$ was suggested (13). Other data which have been obtained are (5): pK_a 5.6, equivalent weight 710 in 60% dimethylformamide; ultraviolet absorption*, λ_{max} 212, 247, 250, and 286 mμ, $E^{1\%}_{1\ cm}$ 337.3, 516.1, 655.0, and 224.4; infrared absorption, λ_{max}** 2.92, 5.80, 5.85, 5.92, 6.06, 6.15, 8.00, 8.44, 8.59, 9.01, 9.09, 10.93, and 12.93 μ; analytical data, C, 64.26, H, 8.37 (average of two determinations). It is interesting to note that the analytical data and the X-ray molecular weight determination are consistent with the formula $C_{44}H_{68}O_{14}$ (molecular weight, 821): calculated C, 64.37; H, 8.35.

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* The solvent was not stated.

** The method of determination was not stated.

Purpose of This Research

The goal of this research was to determine as many of the structural features as possible of the potent, toxic antibiotic flavensomycin. Flavensomycin has several unusual properties which make a study of this type particularly interesting. Based on our results, it is possible that flavensomycin is a macrocyclic lactone (macrolide).

The chemistry of the macrolide antibiotics such as erythromycin, magnamycin, and others has been extensively studied. Generally, the macrolide antibiotics are both antifungal and antibacterial substances that do not have significant ultraviolet chromophores. However, the polyene macrolides such as nystatin, filipin, and candidin are only antifungal and have very characteristic ultraviolet absorption.

Although flavensomycin has a strong ultraviolet chromophore, the absorption is not at all typical of the polyene antibiotics. Many of the macrolide antibiotics contain unusual sugar components, several of which are amino sugars. On the other hand, methanolysis of flavensomycin produces the unusual compound, flavensomycinic acid (XIII).

It thus seems likely that flavensomycin is a new type of antibiotic, possibly intermediate between the true macrolide and the polyene family of antibiotics. Thus knowledge of the structure of flavensomycin will add to data on structure-activity-toxicity relationships, particularly in the macrolide-polyene group of antibiotics.

CHAPTER II

EXPERIMENTAL

Apparatus and Techniques

Dry methanol refers to material that had been distilled from magnesium methoxide. Stock methanol was always distilled before use. Carbon tetrachloride was distilled from calcium chloride. Anhydrous ether was purchased (Merck reagent 71633) and stored over sodium ribbon. Benzene was redistilled, but it was not stored over sodium ribbon. Petroleum ether was always distilled before used. Anhydrous pyridine was prepared by repeated distillation of purified pyridine (Matheson, Coleman and Bell PX 2025) from potassium hydroxide pellets. Pyridine purified in this manner did not become yellow when stored over potassium hydroxide pellets. Acetic acid (Fisher, A-38) was always distilled before use. Dowex-50-X8 (100-200 mesh, Baker reagent 1930) ion-exchange resin was stirred with 4 N hydrochloric acid for several hours and washed with distilled water until the washings were free of chloride. The resin was then washed repeatedly with dry methanol and dried *in vacuo* overnight. All pH measurements, unless otherwise stated, were made using Hydrion paper (Micro Essential Laboratory). Unless otherwise stated, flavensomycin was used as received.

All melting points were determined using a Köfler hot stage and are corrected. Microanalyses were performed by Galbraith Laboratories (Knoxfille, Tennessee), Bernhardt Laboratories (Mülheim, West Germany),

and Geller Microanalytical Laboratories (Charleston, West Virginia).

The drying of solutions in organic solvents was accomplished by the addition of anhydrous magnesium sulfate (Mallinckrodt AR 6070). Gravity filtration was used to remove the drying agent, and it was always washed with several portions of the solvent. Solutions so obtained were evaporated by the use of a Rinco (Model VE-1000-A) rotating evaporator at water aspirator vacuum (acetic acid was evaporated using an oil pump).

All infrared spectra were determined using a Perkin Elmer Model 137 Infracord recording spectrophotometer. Potassium bromide was used for all pellet spectra. Generally about one milligram of the compound was mixed with ca. 200 mg. of potassium bromide using a mortar and pestle. This mixture was then pressed into a pellet using about 60,000 p.s.i. pressure. All ultraviolet spectra were determined using a Cary Model 14 recording spectrophotometer. Optical rotations were determined using a Bellingham and Stanley Polarimeter. The sodium D line was used. All n.m.r. spectra were determined using a Varian Model A-60 spectrometer. The magnet temperature was not measured, but has been shown to be essentially constant (15). In all cases either tetramethylsilane (TMS) (organic solvents) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (deuterium oxide) was used as an internal standard.

Gas liquid chromatography (GLC) was performed using a Glowall Corp. Chromalab Model A-110 instrument equipped with Minneapolis Honeywell continuous recorder (50 mv full scale). In all cases an argon ionization detector was used; the flow rate was monitored using a ten-milliliter buret (soap bubble technique). A power Proportioning Temperature Programmer (Model 240) was used to control and monitor the column temperature

(isothermal and temperature programmed). All solid supports and liquid phases were purchased from Applied Science Laboratories, State College, Pennsylvania. Glass columns (4 mm. o.d., length as stated in text) shaped in the form of a helix, were either purchased from Applied Science Laboratories or constructed locally.

It was necessary to pretreat the solid support. Approximately 100 g. of Chromosorb W (100/120 mesh) was mixed with ca. 500 ml. of concentrated hydrochloric acid. The mixture was stirred occasionally during several hours and allowed to stand overnight. It was then filtered on a large sintered glass funnel and washed several times with distilled water. The support was then placed in a large (ca. 4 ft. x 3 in.) chromatography column, and back-washed for about 30 min. using a slow stream of tap water. This treatment removed the "fines." The support was collected and dried in an oven at 120°. The dry support was then mixed with one liter of two per cent dichlorodimethylsilane in benzene in a two-liter round-bottomed flask. The mixture was swirled while applying vacuum with a water aspirator for brief intervals. This was continued until air bubbles no longer formed. After standing for ca. 15 min., the mixture was filtered, and the support was washed repeatedly with methanol. It was dried in an oven at 120° and then sieved through an 80-mesh screen.

SE-30 stationary phase was applied to the above support by a "solution coating" technique (16). For the preparation of a three per cent SE-30 packing, a solution of three grams of SE-30 in 100 ml. of distilled toluene was used. The solution was mixed with 20 g. of support in a 500-ml. round-bottomed flask and swirled while applying a vacuum with a water aspirator for brief intervals. This was continued until air bubbles no

longer formed. This mixture was then filtered on a sintered glass funnel; a water aspirator vacuum was applied until no more liquid drained from the support (the product was not washed). The coated support was then placed on a large piece of aluminum foil (done in a hood) and spread into a very thin layer. With almost constant mixing, using a small wire brush, the support was allowed to dry at room temperature. The product was then dried at 120° for ca. two hours. The actual per cent of liquid phase on the solid support was determined by eluting a known amount of the packing with boiling benzene on a tared sintered glass funnel. The weight of the support was then determined after it had been dried at 120°. In one particular case, the use of a three per cent SE-30 in toluene resulted in a 2.9% coated support.

Prior to packing, the glass column was filled with concentrated nitric acid. After ca. 30 min., the nitric acid was drained, and the column was washed thoroughly with water and then with absolute ethanol. After the column had been dried at 120°, it was filled with a two per cent solution of dimethyldichlorosilane in benzene. After standing for ca. 15 min., the column was drained, washed with absolute ethanol, and dried at 120°. Glass wool used in conjunction with the column was silanized using the above solution.

A one-half inch plug of glass wool was inserted into the bottom of the column, which was then connected to a water aspirator. The coated support was then slowly added through the top of the column with constant tapping. The column was filled to within one inch of the argon inlet tube and a glass wool plug was inserted.

All columns were cured by programming the temperature (10°/min.)

to 300°; the column was allowed to remain at this temperature for three hours (detector disconnected, no argon flowing). The temperature was then lowered to 250° and maintained at this value for 24 hr. (detector disconnected, argon flow rate ca. 10-20 cc./min.).

For the SE-30 columns prepared during this study, the number of theoretical plates was calculated in the usual way (18), using methyl ester standards. The average of several determinations gave about 1000 theoretical plates per foot for the six and twelve-foot columns, and about 800 theoretical plates per foot for the thirty-foot column.

The DC-QF-1 Column was prepared as described elsewhere (17).

The measurement of peak areas was performed using a Gelman Instruments Co. (Model 39231) planimeter. Semi-log plots of carbon number versus log retention time were constructed for homologous series of compounds (18). These plots were linear if the retention times were measured from the solvent front (initial recorder response). Silicic acid and alumina columns were prepared as described elsewhere (19). Thin-layer chromatography (TLC) was performed as described previously (20).

Molecular weights were determined using a Mechrolab Vapor pressure osmometer (Model 361 A). In all cases the instrument was thermostated at 37°. The same lot of solvent was used for the calibration solute (benzil) and the solute under investigation. Values of ΔR (ΔR is the difference in resistance between the two thermistor beads, and is thus proportional to the molality, m, of the sample solution) were recorded 4.5 min. after the solute solution was placed on the appropriate thermistor bead.

Mass spectrometry (MS) (that which was not done in conjunction

with GLC) was done at the University of Illinois by the use of an Atlas-MAT Mass Spectrometer CH4. For ionization of the samples a TO 4 thermionic/furnace ion source was used; an Atlas Vacuum lock was used in conjunction with the introduction of samples.

Mass spectra that were determined in conjunction with gas-liquid chromatography (GLC-MS) were obtained using a LKB Model 9000 Gas Chromatography-Single Focusing Mass Spectrometer. The ion source was of the electron bombardment type, employing a Rhenium filament. A jet-type molecular separator, of Becker-Ryhage design, was used to increase the ratio of sample to carrier gas. These spectra were obtained through the courtesy of Dr. C. C. Sweeley, University of Pittsburgh, Pittsburgh, Pennsylvania.

Purification and Properties of Flavensomycin

Source and Purity of Flavensomycin as Received

The flavensomycin used in this study was produced by the microorganism *Streptomyces griseus*. The observation that this strain produced flavensomycin was first made at the University of Illinois (3). The sample obtained was a gift of the Upjohn Company, where it was produced and purified.

The material was isolated by extraction of the culture medium with ethyl acetate followed by two 1000 transfer equilibrations in a Craig counter-current distribution apparatus. The theoretical distribution curve was fitted with K = 0.50 (solvent system: ethyl acetate, cyclohxane, 95% ethanol, and water 2:4:5:1). TLC (silica gel HF_{254}, 20% methanol in ether, visualized using both iodine vapor and ultraviolet light)

showed R_F 0.00 (vw), 0.34 (s), and 0.87 (vw). Visual estimation of the intensities of the TLC spots indicated the material was ca. 95% pure. Spectroscopic and physical properties (except m.p.) for the purified (cf. p.21) and unpurified material were essentially identical.

A sample of unpurified flavensomycin was analyzed (dried at 50° in vacuo).

Anal. $C_{47}H_{69}NO_{14}$ (872.07)

Calc'd: C, 64.73; H, 7.98; N, 1.61; O, 25.68;
$C-CH_3$ (6.51 groups), 11.20%;
$O-CH_3$ (2.62 groups), 9.34%;
$N-CH_3$ (0.26 groups), 0.86%;
Active H (7.45 groups), 0.855%;
Lactone titration (four groups consuming base), eq. wt. 218.

Found : C, 64.40; H, 8.02; N, 2.16; O, 25.32;
$C-CH_3$, 11,10, 11.29%;
$O-CH_3$, 9.34%;
$N-CH_3$, 0.86%;
Active H, 0.855%;
Lactone titration, eq. wt. 216.

Chromatographic Approaches to the Purification of Flavensomycin

Alumina Chromatography. A 1.000-g. sample of flavensomycin was chromatographed on acid washed alumina (25 g., column 8.0 cm. x 3.4 cm.) using one per cent methanol in benzene (fraction volume 150 ml.). Fractions 1-4 (yellow oil), 5-6 (yellow solid), and 7-8 (yellow solid) weighed 19.7, 551.2, and 60.8 mg., respectively. TLC (silica gel G, 20% methanol in ether, visualized using iodine vapor) of fractions 1-4 showed R_F 0.93; fractions 5-6 showed R_F 0.32 (s), and 0.90 (vw); fractions 7-8 showed R_F 0.30. Under the same conditions flavensomycin showed R_F 0.00 (vw), 0.30 (s), and 0.91 (vw).

A 202-mg. sample of flavensomycin (from fractions 5 and 6) was chromatographed on acid washed alumina (5.0 g., column 6.0 cm. x 1.2 cm.) using 1% methanol in benzene as the eluting solvent. Elution was continued until no more material came off the column. This yielded 160.9 mg. of material (80%). TLC (silica gel HF_{254}, 25% methanol in ether, visualized using ultraviolet light) showed R_F 0.39 (s) and 0.95 (vw). The alumina remaining in the column had a bright yellow color.

Attempted Silicic Acid Chromatography. A 102-mg. sample of flavensomycin was chromatographed on silicic acid (ca. five grams, column 5.5 cm. x 1.2 cm.). Elution was started with benzene (fraction volume 20 ml.). Fractions 1-3 contained no material. Fractions 4-6 (1% methanol in benzene) weighed 61.3, 17.6, and 8.9 mg., respectively. Fraction 7 (ethyl acetate) weighed 16.3 mg. TLC of fraction 4 (silica gel, HF_{254}, 25% methanol in ether, visualized using ultraviolet light) showed R_F 0.38 (s), 0.95 (w), and of fraction 5 showed R_F 0.00 (m), 0.38 (s), and 0.95 (m). Under the same conditions flavensomycin showed R_F 0.00 (vw), 0.36 (s), and 0.93 (vw).

A 5.00-g. sample of flavensomycin was chromatographed on silicic acid (200 g., column 22 cm. x 5.6 cm.). The column was prepared using benzene; a linear gradient elution with one liter solvent reservoirs was employed (21). Elution was started with benzene versus two per cent methanol in benzene (total volume two liters). This did not elute any material. The elution solvent was then changed to two per cent methanol in benzene versus four per cent methanol in benzene (fraction 1, wt. 2.5838 g.) (total volume, two liters), then four per cent methanol in benzene (fraction 2, wt. 1.0295 g.) total volume, two liters), and finally ethyl acetate (fraction 3, wt. 0.8791 g.) (volume, one liter) (total wt. 4.4924 g., 89.8% recovery). TLC (silica

gel HF_{254}, 20% ethanol in ether, visualized using ultraviolet light) of fraction 1 showed R_F 0.00 (vw), 0.10 (m), 0.31 (s), and 0.85 (m); fraction 2 showed R_F 0.00 (w), 0.12 (m), 0.31 (m), and 0.90 (m); fraction 3 showed R_F 0.00 (s), 0.32 (w), and 0.90 (vw). Under the same conditions, flavensomycin showed R_F 0.00 (vw), 0.30 (s), and 0.89 (vw).

Part of fraction 1 (254 mg.) was recrystallized from benzene (10 ml.) and n-hexane (30 ml.). This yielded 43.5 mg. of a finely divided yellow powder. TLC (silica gel HF_{254}, 20% methanol in ether, visualized using ultraviolet light) showed R_F 0.00 (w), 0.40 (s), 0.95 (m). Under the same conditions, recrystallized flavensomycin showed R_F 0.41.

Fraction 3 was shown to be mainly flavensomycinoic acid (cf. p. 36).

Crystallization of Flavensomycin

For analysis, 130 mg. of flavensomycin (from fractions 5-6 of the alumina column, cf. p. 19) was dissolved in four milliliters of warm benzene and four milliliters of warm n-hexane was added. The solution was centrifuged, and the clear supernatant liquid was transferred. The solution was then allowed to cool to room temperature and was then cooled in a refrigerator overnight. The crystalline material was collected and dried under high vacuum at 50°C (yield, 43.2 mg.). This material showed m.p. 141-143.5°, with sweating at ca. 120°, darkening at ca. 130°, resolidification at 145-146°, and gradual remelting at ca. 190-210°. TLC (silica gel G, 20% methanol in ether, visualized using iodine vapor) showed R_F 0.31. The ultraviolet spectrum of this substance showed λ_{max} 250, ca. 275 (sh.) mμ, ε = 52,000, and 25,000 (95% ethanol) and is shown in part as Figure 6. The infrared spectrum (pellet) is shown as Figure 1.

Attempts to measure the optical rotation of flavensomycin were not successful; the intense yellow color of the solution did not allow enough light to pass through. The n.m.r. spectrum of flavensomycin (30% deuteriochloroform) is shown as Figure 2. The relative areas of various regions of the n.m.r. spectrum of flavensomycin were determined using a planimeter. The results are shown below.

Range or Position (τ)	Area (in.^{2})	No. of Protons (Total = 69)
-3.50	0.25	.99
0.83	0.23	.91
2.33-3.83	0.92	3.62
3.83-5.58	1.38	5.43
5.58-7.00	3.06	12.05
7.00-7.67	1.75	6.89
7.67-8.50	3.54	13.94
8.50-9.67	6.39	25.17

The mass spectrum is shown as Figure 3.

Anal. $C_{47}H_{69}NO_{14}$ (872.07) Calc'd: C, 64.73; H, 7.98; N, 1.61; O, 25.68
Found : C, 65.17; H, 8.07; N, 1.76; O, 25.03

Molecular Weight of Flavensomycin

In order to determine the molecular weight of flavensomycin in absolute ethanol (benzene was unsatisfactory because of association), it was necessary to prepare a calibration curve using that solvent. Benzil

(recrystallized twice from ethanol, m.p. 94.6-95.8°) was used as the standard. Seven solutions of benzil in absolute ethanol were prepared gravimetrically in glass-stoppered 25-ml. Erlenmeyer flasks. The solutions ranged in concentration from 0.00206 to 0.0980 molal. The calibration curve was constructed by plotting values of $\Delta R/m$ versus ΔR. Two different samples of flavensomycin were determined. One sample was part of fractions 5 and 6 from an alumina chromatography column (cf. p. 19). The other sample was part of that same fraction that had been recrystallized from benzene-n-hexane.

The values obtained for flavensomycin, as well as other known compounds, are shown in Table 1. Each value represented an average of from two to five determinations.

Table 1. Molecular Weights by Vapor Pressure Osmometry

Sample	MW	Determined MW
Flavensomycin, column fraction	872*	846
Flavensomycin, recrystallized	872*	849
Dicyclohexylurea	223	229
3-Chloro-6-hydroxyacetophenone	170.5	182
1-Docosanol	326	334
Docosanoic acid	340	365
Usnic acid	344	340

* Determined by mass spectrometry.

Solvent Stability of Flavensomycin

Solutions of two milligrams of recrystallized flavensomycin in 0.5 ml. of the following solvents were prepared: 1, chloroform; 2, carbon tetrachloride; 3, benzene; 4, methanol; 5, methanol-water (1:1); 6, pyridine; 7, dioxane; 8, acetone; 9, acetic acid; 10, methylene chloride; 11, ethyl acetate; 12, tetrahydrofuran.

The solutions were allowed to stand at room temperature and were not protected from light. The TLC results (silica gel HF_{254}, 25% methanol in ether, visualized using ultraviolet light) at various times are summarized in Table 2. In another study (stock flavensomycin), using benzene and chloroform as solvents, the results shown in Table 3 were obtained by the use of TLC (silica gel G, 30% methanol in ether, visualized using iodine vapor).

Chloroform Degradation of Flavensomycin. A 27.5-mg. portion of flavensomycin was dissolved in 20 ml. of chloroform, and the solution was boiled under reflux for two days. The solvent was evaporated, and the residue was chromatographed over unisil (Clarkson Chemical Co., Inc., 200-300 mesh) (5 g.). Elution with benzene (10 ml., fraction 1) yielded 7.5 mg. of a white solid. Elution with 2% methanol in benzene (10 ml., fraction 2) gave 15.0 mg. of a yellow solid. Further elution with 6% methanol in benzene (10 ml., fraction 3) yielded 3.4 mg. of a yellow solid. TLC (silica gel HF_{254}, 3:1 methanol-benzene, visualized with ultraviolet) showed: fraction 1, R_F 0.00 (w) and 0.90 (s); fraction 2, R_F 0.00 (w), 0.36 (s); fraction 3, R_F 0.00. Under the same conditions flavensomycin showed R_F 0.32.

The ultraviolet spectra in 95% ethanol showed: fraction 1, λ_{max}

Table 2. Stability of Flavensomycin in Various Solvents as a Function of Time. R_F Values.

	Solvent*											
Time	1	2	3	4	5	6	7	8	9	10	11	12
One hour	0.42	0.42	0.41	0.40	0.41	0.42(s) 0.90(m)	0.44	0.43	0.00(s) 0.43(m) 0.92(s)	0.40	0.43	0.42(s)
One day	0.45	0.44	0.44	0.42	0.42	0.00(m) 0.42(w) 0.90(s)	0.45	0.46	0.00(s) 0.44(vw) 0.92(s)	0.42	0.43	0.00(w) 0.42(s) 0.93(m)
One week	0.00(m) 0.44(m) 0.92(m)	0.00(w) 0.45(s) 0.91(w)	0.00(vw) 0.43(s) 0.93(vw)	0.00(w) 0.44(s) 0.93(w)	0.00(w) 0.44(s) 0.94(m)	0.00(s) 0.91(s)	0.00(w) 0.46(s) 0.90(w)	0.00(w) 0.44(s) 0.91(w)	0.00(s) 0.93(s)	0.00(m) 0.42(m) 0.94(m)	0.00(m) 0.44(m) 0.91(m)	0.00(m) 0.45(w) 0.93(s)

* The composition of solvents 1-12 are given on p. 24.

225 and 312 mμ ($E_{1\ cm}^{1\%}$ 170 and 14.7); fraction 2, λ_{max} 248 and 280 (sh.) mμ ($E_{1\ cm}^{1\%}$ 355 and 123); fraction 3, λ_{max} 220, 258, and 325 mμ ($E_{1\ cm}^{1\%}$ 425, 351, and 171).

Ultraviolet Spectra Flavensomycin in 1 N Ethanolic Hydrochloric Acid

A solution was prepared from 38.0 mg. (0.0436 mmole) of flavensomycin, 4.17 ml. (50 mmole) of concentrated (12 N) hydrochloric acid, and enough 95% ethanol to make the total volume 50 ml. (the solution was therefore 1 N in hydrochloric acid). Two milliliter aliquots of the solution were taken at various times, diluted to 100 ml. with 95% ethanol, and the ultraviolet spectra were determined. The results are summarized in Table 4.

Attempted Sodium Borohydride Reduction of Flavensomycin

A solution of 210 mg. (0.242 mmole) of flavensomycin and 210 mg. (5.56 mmole) of sodium borohydride in 15 ml. of absolute ethanol was allowed to stand in the dark at room temperature for three days. The ethanol was evaporated, and 30 ml. of benzene containing 333 mg. (5.56 mmole) of acetic acid was added. The layers were separated, and the aqueous layer (pH = 5.0) was extracted with two 15-ml. portions of benzene. The combined benzene layers were dried and evaporated, yielding a pale yellow oil. The oil was dissolved in benzene and lyopholyzed; this gave a pale yellow solid (160 mg.). The solid was non-crystalline and showed m.p. darkening ca. 120°, melting ca. 145°. The infrared spectrum (pellet) of this substance showed λ_{max} 2.88, 3.38, 5.85, 6.17, 7.12, 7.37, 7.52, 8.07, and 8.21 μ, among others. The ultraviolet spectrum (95% ethanol) showed λ_{max} 250 and 273 mμ, $E_{1\ cm}^{1\%}$ 580 and 498. The $E_{1\ cm}^{1\%}$ values for flavensomycin at 250 and 273 mμ were 596 and 284, respectively. The n.m.r.

Table 3. Stability of Flavensomycin in Benzene and Chloroform.

Solvent	Conditions	R_F Values
Benzene	Zero time	0.00(vw), 0.54(s), and 0.96(vw)
Benzene	Boil under reflux for two days	0.00(w), 0.55(s), and 0.97(w)
Chloroform	Stand overnight	0.00(w), 0.55(s), and 0.97(w)
Chloroform	Boil under reflux overnight	0.00(s) and 0.97(s)

Table 4. Ultraviolet Spectra of Flavensomycin in 1 N Ethanolic Hydrochloric Acid.

Time (hr.)	λ_{max}	ε	λ_{max}	ε
In pure 95% ethanol	250	52,000	≈ 275*	25,000
0.0	248	48,600	≈ 280*	22,500
1.0	246	45,300	≈ 270*	30,100
3.0	243	39,600	≈ 265*	29,800
4.5	242	38,700	≈ 265*	30,800
30	234	33,400	267	32,100
54	230	36,800	268	33,600
72	229	36,700	268	33,600
96	232	28,400	268	38,000
120	235	27,600	268	37,600
168	243	26,500	268	29,700

* shoulder

spectrum (ca. 30% in deuteriochloroform) showed absorption at (all areas of absorption were broad, and many were complex) 3.7 (1)*, 4.45 (1), 6.1 (2), 6.5 (3), 6.9 (4), 8.1 (6), 8.75 (6), and 9.1 τ (10). TLC (silica gel HF_{254}, 4% methanol in chloroform, visualized using iodine vapor and ultraviolet light) showed R_F 0.00 (m), 0.10 (m), 0.19 (w), 0.38 (s), and 0.48 (m). Under the same conditions flavensomycin showed R_F 0.00 (s) with streaking to ca. 0.30 (vw).

The aqueous layer was lyopholyzed and gave a pale yellow, glassy solid. This solid was powdered with a glass rod and triturated with ca. 20 ml. of boiling ethyl acetate. The ethyl acetate was separated and evaporated. This yielded no material. The solid was then triturated with ca. 20 ml. of boiling benzene-methanol (5:1), and the liquid was separated. Evaporation of the solvents yielded only traces of material, which did not burn completely in a flame.

Methanolysis of Flavensomycin

A mixture of 500.5 mg. of flavensomycin, 50 ml. of dry methanol, and 253 mg. of dry Dowex-50-X8 was magnetically stirred. The reaction was followed by TLC (silica gel G, 20% methanol in ether, visualized using iodine vapor); the results are summarized in Table 5.

After four days, the methanolic mixture was brought to boiling, and the Dowex-50-X8 was filtered from the solution. The Dowex-50-X8 was washed with two 10-ml. portions of boiling methanol. On evaporation of the methanol a yellow, partially crystalline mass was obtained. This

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* The values in parentheses are the relative areas of the peaks obtained by estimating the number of squares enclosed by that peak; 1 is the smallest, 10 is the largest.

Table 5. Methanolysis of Flavensomycin.

Time (days)	R_F Values			
0.0	0.00(vw),		0.34(s),	0.86(vw)
0.5	0.00(w),		0.34(s),	0.87(w)
2.0	0.00(s),	0.09(m),	0.35(m),	0.89(s)
3.0	0.00(s),	0.08(m),	0.33(w),	0.86(s)
4.0	0.00(s),	0.09(m),	0.42(w),	0.87(s)

material was divided into three fractions; hexane-soluble* 384.7 mg., benzene-soluble 46.7 mg., and the residue 73.3 mg. (total weight 504.7 mg.). TLC (silica gel G, 20% methanol in ether, visualized using iodine vapor) showed: hexane-soluble, R_F 0.00(w), 0.55(m), and 0.90(s); benzene-soluble, R_F 0.00; residue, R_F 0.00.

Alumina Chromatography of Hexane-Soluble Fraction. The hexane-soluble material (384.7 mg.) was chromatographed on acid washed alumina (50 g., column 29 cm. x 1.5 cm.). Elution was started with benzene (fraction volume 100 ml.). Fractions 1-5 (benzene) contained no material. Fractions 6-8 (1% methanol in benzene) weighed 28.4, 108.6, and 8.0 mg., respectively, and could be lyopholyzed to cream colored solids. An infrared spectrum (4% in carbon tetrachloride) of fraction 7 showed λ_{max} 2.85, 3.36, 5.83, 5.92, 6.19, and 6.89 μ, among others. TLC of fraction 7 (silica gel G, 20% methanol in ether, visualized using iodine vapor)

* The solubility of this fraction in hexane may have been due to some residual methanol.

R_F 0.00(s), 0.08(m), 0.16(m), and 0.25(s). Further elution of the column (pure methanol) yielded 71.4 mg. of material which was not investigated. The total recovery was 284.9 mg. (76%).

Catalytic Reduction of Flavensomycin

A number of reductions of flavensomycin were performed according to the following general procedure. In general, the ratio of flavensomycin to solvent was about 100 mg. to 10 ml. In one-half of the total solvent to be used, five per cent platinum on carbon was equilibrated with hydrogen in a room temperature, atmospheric pressure hydrogenation apparatus. Stirring was stopped, and the sample of flavensomycin (dissolved in one-half of the total solvent) was added to the reaction flask. The reading on the buret was recorded, and the hydrogenation was commenced by starting the magnetic stirrer. Buret readings were taken at various times until the uptake of hydrogen had ceased.* The catalyst was filtered from the solution through a celite mat. The celite was washed with several portions of fresh solvent, and the total filtrate was evaporated to dryness (when acetic acid was used the filtrate was sometimes lyopholyzed). This yielded a colorless glass, which in some cases was partially crystalline. The data on runs 1-6 are summarized in Table 6. A plot of the ratio of moles of hydrogen per mole of flavensomycin versus time for run 6 (not shown) revealed that the reaction proceeded at almost constant rate up to a ratio of ca. 4 (time 1.75 hr.). The reaction then proceeded much slower; ratios of 5 at 2.3 hr., 6 at 3.1 hr., and finally 6.92

* In two reactions the uptake of hydrogen was stopped at ca. five moles/mole.

Table 6. Catalytic Reduction of Flavensomycin.

Run No.	Solvent	Wt. Catalyst (mg.)	Wt. Flavensomycin (mg.)	Wt. Product (mg.)	Time Required for Reduction (hr.)	Ratio*
1	Acetic acid	206.0	200.2	169	ca. 10	7.1
2	Acetic acid	ca. 500	500.0	511	3.1	5.25**
3	Acetic acid	ca. 500	1003.2	994.9	5.7	5.10**
4	Ethanol	500	1000.0	916.6	ca. 12	3.8
5	Acetic acid	105	103.5	100.5	5.0	6.9
6	Acetic acid	2010	2002	2004	6.0	6.92

* Moles of hydrogen per mole of flavensomycin.

** Stopped at this ratio (ca. five moles/mole).

at 6.0 hr. were observed.

Isolation of Dihydroflavensomycinoic Acid. The product from one particular hydrogenation of flavensomycin in acetic acid (2.004 g., run No. 6) was chromatographed over silicic acid (20 g., column 9.5 cm. x 2.2 cm.) using chloroform. The first 300 ml. (fraction 1) eluted 1.553 g. of a colorless glass (which will be called the perhydro fragment). The elutant was changed to 5.0% methanol in chloroform (fraction volume, 25 ml.). Fractions 2-5 contained 65 mg. of an oily, yellow material that contained a small amount of solid. These fractions were discarded. Fractions 6-12 contained 0.395 g. of a crystalline product. This material was dissolved in the minimum amount of warm chloroform-methanol (5:1) and centrifuged. The clear supernatant liquid was removed from some insoluble material. The solution was heated to boiling and carbon tetrachloride was added until crystallization began. After cooling to room temperature, the product was isolated by filtration, yielding 0.345 g. (0.00150 mole, 70% based on 0.00229 mole of flavensomycin) of white, fluffy crystals. The product, when heated on a hot stage, began to decompose at ca. 160-170° and at ca. 180° melted with decomposition and subsequent resolidification.

TLC (silica gel G, benzene-methanol-acetic acid (45:8:4), visualized with iodine vapor) of this material showed R_F 0.40. An infrared spectrum (pellet) of the compound showed λ_{max} 3.07, 3.23, 3.35, 3.76, 5.82, 5.98, and 6.30 μ, among others. The n.m.r. spectrum (11% in pyridine, w/v) of the compound showed absorption at -3.10 (2H, singlet), -0.27 (1 H,* broad singlet), 7.00 (4H, multiplet), and 7.66 τ (4H,

* Assumed.

singlet).

For analysis, a small portion of the compound was recrystallized in the same manner. It showed the same melting point behavior.

Anal. $C_9H_{11}NO_5$ (213.2) Calc'd: C, 50.71; H, 5.20; N, 6.57; $C\text{-}CH_3$, 0.00%; $O\text{-}CH_3$, 0.00%; $N\text{-}CH_3$, 0.00%

Found : C, 50.92; H, 5.35; N, 6.61; $C\text{-}CH_3$, 2.17%; $O\text{-}CH_3$, 0.00%; $N\text{-}CH_3$, 0.00%

The product from another hydrogenation (run No. 4, 916.6 mg.) was chromatographed on silicic acid. Elution with chloroform yielded 845.8 mg. of a glass. Elution with 3.0% methanol in chloroform yielded 87 mg. of the crystalline product.

Purification of the Perhydro Fragment. A 1.262 g. portion of the perhydro fragment (cf. p. 32) was chromatographed on acid washed alumina (120 g., column 31.5 cm. x 2.2 cm.) (fraction volume 200 ml.). Fractions 1-5 (benzene eluant), 6-10 (50% chloroform in benzene eluant), and 11-16 (chloroform eluant) weighed 62.7, 163.2, and 1029.3 mg., respectively (total weight, 1255.2 mg.). TLC of the crude material (silicic gel HF_{254}, four per cent methanol in chloroform, visualized using both iodine vapor and ultraviolet light) showed R_F 0.00(s), streaking to 0.70(w), and 0.83(s); fractions 1-5 R_F 0.84; fractions 6-10 R_F 0.81; fractions 11-16 R_F 0.82. An infrared spectrum (carbon tetrachloride solution) of fractions 11-16 showed λ_{max} 2.78, 3.37, 5.74, 6.84, 7.25, and 7.93 μ, among others. The n.m.r. spectrum (ca. 50% in carbon tetrachloride of this fraction showed poorly resolved absorption at 6.7 (1.5),*

* See footnote, p. 28.

8.6 (7), and 9.1 τ (10). GLC (six-foot column, 10% QF-1, CT 246°, AIP 20 psi) of the trimethylsilyl derivative of the perhydro fragment showed peaks at 14.2, 17.5, 18.9, 20.7, 26.2, 28.2, 29.8, and 31.3 min. with areas of 0.69, 7.74, 1.80, 6.67, 1.62, 0.57, 0.71, and 0.12 $in.^2$, respectively.

Further Characterization of Dihydroflavensomycinoic Acid and Flavensomycinoic Acid

Dihydroflavensomycinoic Acid

Ultraviolet Spectrum as a Function of pH. A 1.716-mg. (0.00805 mmole) sample of the acid was dissolved in 50 ml. of water. Buffer solutions were prepared at twice their normal concentration in the following manner: pH 1.0, hydrochloric acid; pH 2.0 and 7.0, Coleman "buffer tabs" (Fisher 11-505-238); pH 3.0, 4.0, 5.0, and 6.0, citric acid and disodium phosphate (22). Solutions for ultraviolet determinations were prepared by mixing 2.00 ml. of the dihydroflavensomycinoic acid solution with 2.00 ml. of the buffer solution. The reference solvent was prepared by mixing 2.00 ml. of water with 2.00 ml. of buffer solution. The results are given as Figure 4.

Potentiometric Determinations of the pK_a Values. A sample of dihydroflavensomycinoic acid (5.370 mg., 0.0252 mmole) was dissolved in 2.90 ml. of distilled water. To this solution was added 100 µl. of 1.225 N hydrochloric acid. The solution was titrated with 1.000 N potassium hydroxide; the pH of the solution was determined after each increment of base was added using a Beckman Zeromatic pH meter. Increments of base were ten microliters when the pH was not changing rapidly and one

microliter when the pH was changing more rapidly. A blank solution containing 2.90 ml. of distilled water and 100 µl. of 1.225 N hydrochloric acid was titrated as described above. The pK_a values for flavensomycinoic acid were determined by calculating the equivalents of hydrogen ion bound and plotting this value versus pH (23). By fitting a theoretical curve to the experimental curve the pK_a values of dihydroflavensomycinoic acid were determined to be 3.07 and 4.66 [lit. (8) pK_a 2.8 and 3.6 (water)]. To determine the accuracy of this method, nicotinic acid was titrated in a similar manner. The pK_a values determined were 2.37 and 4.70 [lit. (24) pK_a 2.07 and 4.81 (water)].

Attempted Preparation of a 2,4-Dinitrophenylhydrazone Derivative. A 20-mg. portion of dihydroflavensomycinoic acid and five milliliters of 30% aqueous perchloric acid containing 60 mg. of 2,4-dinitrophenylhydrazine were allowed to stand at room temperature for 18 days. This yielded 19.7 mg. of a red solid. The remaining solution was then heated at 90° for 24 hr. This yielded 15.6 mg. of a similar appearing material. This material (total weight 35.3 mg.; theoretical for a mono-2,4-dinitrophenylhydrazone is 36.9 mg.) was appreciably soluble only in pyridine and was not soluble in aqueous base. TLC (silica gel G, benzene-methanol-acetic acid 45:8:4, visual) showed R_F 0.00(s, red), 0.58 (m, yellow-orange), and 0.70(m, yellow-orange). Under the same conditions 2,4-dinitrophenylhydrazine showed R_F 0.40 and 0.70. Using a different solvent system (benzene-methanol 2:1) the sample showed R_F 0.00 (s, red) and 0.85(s, broad orange spot). Under the same conditions 2,4-dinitrophenylhydrazine showed R_F 0.68 (yellow). No satisfactory solvent system could be found for preparative TLC of this mixture. Attempted

chromatography of this substance on acid-washed alumina was not successful; no material could be eluted, even with pyridine.

Preparation and Properties of Dimethyl Dihydroflavensomycinoate. An excess of ethereal diazomethane (prepared from N-methyl-N-nitrosourea) was added to a solution of 80.0 mg. (0.375 mmole) of dihydroflavensomycinoic acid (not recrystallized) in ca. 20 ml. of methanol-chloroform (1:1). After standing several hours, the solvents were evaporated. The partially crystalline product was chromatographed on silicic acid using chloroform and gave, after recrystallization from benzene-petroleum ether (b.p. 100-115°), 61.6 mg. (0.256 mmole, 68%) of lustrous, white needles, m.p. 117-118°. An infrared spectrum (pellet) of this compound showed λ_{max} 3.03, 3.26, 3.36, 5.74, 5.82, 5.92, 6.01, 6.13, 6.51, 6.85, and 6.93 μ, among others. The n.m.r. spectrum (12% in deuteriochloroform) of the compound showed absorptions at 2.07 (1H, broad singlet), 5.92 (3H, singlet), 6.30 (3H, singlet), and 7.33 τ (8H, singlet superimposed on a complex multiplet). The ultraviolet spectrum (ethanol) showed λ_{max} 248 mμ (ε 14,050). The mass spectrum of this compound is shown as Figure 5.

A small amount of this compound was recrystallized from benzene-petroleum ether (b.p. 100-115°) for elemental analysis. The analytical sample showed m.p. 120-121° [lit. (8) m.p. 117-118°].

Anal. $C_{11}H_{15}NO_5$ (241.2) Calc'd: C, 54.77; H, 6.27; N, 5.81
Found : C, 54.88; H, 6.39; N, 5.80

Flavensomycinoic Acid

An impure sample of this acid (175 mg.), obtained from attempted silicic acid chromatography of flavensomycin (cf. p. 20, part of fraction 3) was recrystallized from n-butanol. This yielded 118 mg. of a bright

yellow solid that showed m.p. 252-254° dec. [lit. (9) m.p. 249-251° dec.]. An infrared spectrum (pellet) of this compound showed λ_{max} 2.86, 3.04, 3.21, 3.38, 3.69, 5.86, 6.02, 6.22, and 6.40 μ, among others. The n.m.r. spectrum (saturated in dimethyl sulfoxide-d_6) showed absorptions at 0.10 (broad singlet), 2.56 (doublet, J = 15.0 cps), 3.33 (doublet, J = 15.0 cps), and 7.50 τ (broadened singlet). The ultraviolet spectrum (ethanol) showed λ_{max} 217, 259, and 320 mμ (ε 14,800, 15,800, and 5,150) and is shown in part as Figure 6. The difference in ultraviolet absorption (ethanol) between flavensomycin and flavensomycinoic acid is shown as Figure 6.

Nitric Acid Oxidation. A 43.0-mg. (0.204 mmole) sample of flavensomycinoic acid and 16 ml. of concentrated nitric acid were placed in a 100-ml. three-necked flask equipped with a gas inlet tube and a reflux condenser (ice water was circulated in the condenser). Nitrogen (previously passed through a 50% potassium hydroxide solution) was bubbled through the solution; the exit gases were passed into a saturated barium hydroxide solution. The reaction mixture was heated on a steam bath for a total of four hours.

Determination of carbon dioxide. The precipitated barium carbonate was collected and washed repeatedly with distilled water. The solid was then dissolved in dilute hydrochloride acid, and the barium ion was precipitated using dilute sulfuric acid. The barium sulfate was collected on a tared sintered glass funnel, washed with distilled water, and dried at 140° for two hours. This yielded 360.5 mg. (1.547 mmole) of barium sulfate. This corresponded to 7.49 moles of carbon dioxide from one mole of flavensomycinoic acid.

To test the accuracy of this method, a sample of formic acid (13.15 mmole by titration) was oxidized with 22 ml. of concentrated nitric acid as outlined above. This yielded 3.0649 g. (13.15 mmole, 100%) of barium sulfate.

Determination of volatile acid. The pH of the aqueous portion from the nitric acid oxidation of flavensomycinoic acid was adjusted 1.5 with concentrated sodium hydroxide solution. This solution was distilled to dryness at ca. 40°/20 mm; the collection flask was cooled to 0°. The distillate was made basic (ca. pH 10) with sodium hydroxide solution and lyopholyzed. The solid obtained was dissolved in two milliliters of water, and the pH was adjusted to five using dilute hydrochloric acid. A 55-mg. (0.20 mmole) sample of p-phenylphenacyl bromide (Eastman 3297, recrystallized from benzene, m.p. 126-128°, [lit. (25) m.p. 127°] and 10 ml. of 95% ethanol were added to this solution. This mixture was boiled under reflux for two hours, diluted with 25 ml. of water, and extracted with two 25-ml. portions of chloroform. The chloroform solution was dried and evaporated. This yielded 49.0 mg. of a yellow crystalline solid. GLC (six-foot column, 3% SE-30, CT 196°, AIP 20 psi) of this preparation showed only one peak that had a retention time greater than 7.00 min.; that peak had a retention time of 7.27 min. Under the same conditions the GLC behavior of p-phenylphenacyl acetate showed one peak with a retention time of 7.21 min. Under the same conditions, the product isolated from a blank run (water, ethanol, and p-phenylphenacyl bromide, boiled under reflux for two hours) showed a number of peaks all of which had retention times less than that of p-phenylphenacyl acetate.

Preparation of Standards for Gas Chromatography and Mass Spectrometry

General Procedure for the Preparation of Methyl Esters

Five to twenty-five milliliters of 2,2-dimethoxypropane (Eastman P 7846) was added to a solution of one to five grams of the acid in 5-25 ml. of distilled methanol containing one to five drops of concentrated hydrochloric acid. The solution was allowed to stand for two hours at room temperature; the solvents were then evaporated. The product was dissolved in 20-100 ml. of ether, and an equal volume of water was added. The ether layer was separated, washed with 10-50 ml. of a 10% solution of sodium carbonate, dried, and evaporated. The ester was then transferred to a four-dram vial using benzene, and most of the benzene was evaporated. In none of these preparations was a yield determined, and in only a few was further purification attempted. GLC analysis of the various esters revealed that in no case was there a significant amount of volatile impurity. All of the acids that were converted to methyl esters by this method, as well as by other methods (denoted), are shown in Table 7. The mass spectral data for these esters are summarized in Table 8.

Dimethyl Diethylmalonate

A 3.4-g. (0.021 mole) sample of diethylmalonic acid (prepared by hydrolysis of diethyl diethylmalonate, Eastman 118) was dissolved in ca. 25 ml. of 50% methanol-ether, and an excess ethereal diazomethane (prepared from N,N'-dimethyl-N,N'-dinitrosoterephthalamide) was added. After standing overnight, the solvents were evaporated; the product was transferred to a four-dram vial using benzene. The benzene was evaporated

Table 7. Acids Used to Prepare Methyl Ester Standards

Acid	Obs. m.p.		Lit. m.p.	Reference
Succinic	186-187°		185°	26
Glutaric	94-97°		97.5°	27
Adipic	151-152°		153-153.5°	28
Pimelic	105-106°		105°	29
Suberic	139-141°		140°	30
Azelaic	103-105°		106.5°	31
Sebacic	132-134°		133-133.5°	30
Dodecanedioic	121-124°		123°	32
Tetradecanedoic	120-122°		123°	32
Dimethylmalonic	189-190°		190°	42
Diethylmalonic	124-126°		125°	43
2-Methylsuccinic	119-122°		112.5°	33
2,2-Dimethylsuccinic	145.5-146.5°		142°	34
2,3-Dimethylsuccinic	112-175°	dl	129°	39
		meso	209°	39
meso-2,3-Dimethylsuccinic	206-208°		209°	39
2-Methylglutaric	77.5-79.5°		77-78°	35
3-Methylglutaric	83-86°		85-86°	36
2,2-Dimethylglutaric	83.5-85°		82°	37
2,3-Dimethylglutaric	52-85°	erythro	87°	40
		threo	oil	40
2,4-Dimethylglutaric	108-125°	dl	140-141°	41
		meso	127-128°	41
3-Ethyl-3-methylglutaric	82-83°		82-83°	44
Tricarballylic	158-160°		160°	45
β-Methyltricarballylic	165-167°		164°	46

Table 8. Mass Spectral Data for Known Methyl Ester Standards.

Compound No.	Acid, as Methyl Ester	MW of Ester	RT at 100°*	RT at 136°*	RT at 196°*	Relative Intensities of Peaks at m/e Ξ									
						28	29	30	31	32	33	34	35	36	37
1	Dimethylmalonic	160	4.4			147	18	3	15	85	1	0	0	0	3
2	Succinic	146	5.3			10	7	1	4	5	0	0	0	0	0
3	2-Methylsuccinic	160	6.2			10	9	1	2	3	0	0	0	0	0
4	2,2-Dimethylsuccinic	174	7.2			26	9	1	2	8	0	0	0	0	0
5	meso-2,3-Dimethylsuccinic	174	7.8			227	16	1	3	57	0	0	0	0	1
6	dl-2,3-Dimethylsuccinic	174	8.5			18	13	1	3	3	0	0	0	0	0
7	Glutaric	160	9.0			141	12	2	6	42	0	0	2	1	1
8	Diethylmalonic	188	10.4			26	30	1	5	9	1	0	0	0	1
9	3-Methylglutaric	174	10.7			280	24	3	7	63	1	0	0	0	0
10	2-Methylglutaric	174	11.1			200	32	3	10	50	0	0	0	2	2
11	2,4-Dimethylglutaric	188	11.7			39	18	1	5	7	1	0	1	0	0
12	3,3-Dimethylglutaric	188	12.2			250	20	2	4	71	1	0	0	1	1
13	2,4-Dimethylglutaric	188	13.4			31	16	1	6	5	1	0	1	0	0
14	2,2-Dimethylglutaric	188	14.6			147	11	1	3	34	1	0	0	0	0
15	2,3-Dimethylglutaric	188	15.5			250	28	2	7	55	1	0	0	0	1
16	Adipic	174	15.7	4.8		138	20	2	7	29	1	0	0	0	0
17	3-Ethyl-3-methylglutaric	202	22.4	6.0		140	27	2	6	33	2	0	0	0	0
18	2,5-Dimethyladipic	202	23.5	6.4		16	17	1	4	6	1	0	0	0	0
19	Pimelic	188	29.6	7.3		100	20	2	8	20	2	0	0	0	0
20	Tricarballylic	218		9.4		82	6	3	4	32	1	0	0	0	0
21	β-Methyltricarballylic	232		9.8		36	14	1	5	8	0	1	0	0	0
22	Suberic	202		11.4	3.3	56	24	2	8	12	1	0	0	0	0
23	Azelaic	216			4.2	62	27	1	7	12	1	0	0	0	0
24	Sebacic	230			5.4	54	26	1	5	11	1	0	0	0	0
25	Dodecanedioic	258			9.6	24	32	1	5	10	1	0	0	0	0
26	Tridecanedioic	272			13.1	45	27	1	4	21	1	0	0	0	0
27	Tetradecanedioic	286			18.3	35	19	1	3	16	1	0	0	0	0
28	Stearic	298			24.6	20	13	1	1	5	0	0	0	0	0

* Twelve-foot column, 3% SE-30, FR = 30 cc./min.

Table 8. Continued

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
1	7	40	10	77	32	34	11	11	0	0	0	1	11	14	14	3	1	7	1	4	1	81	1	3
2	0	1	1	5	5	4	2	10	0	0	0	0	0	0	0	1	1	80	8	8	2	60	2	0
3	1	10	3	19	11	8	2	3	0	0	0	0	1	1	1	1	0	9	2	1	2	100	4	1
4	0	10	2	20	2	17	2	2	0	0	0	0	0	0	0	2	1	24	6	2	3	30	0	0
5	1	9	4	15	2	9	4	4	0	0	0	0	1	2	1	4	3	27	16	12	1	100	4	0
6	1	10	2	18	2	9	1	4	0	0	0	0	1	1	0	4	3	30	16	10	1	100	5	1
7	3	15	6	34	31	49	11	6	0	0	0	4	1	2	1	1	1	59	4	5	20	100	3	0
8	2	26	5	63	8	13	2	14	0	0	0	0	1	2	1	11	3	34	2	3	1	100	4	1
9	2	26	6	46	34	54	6	7	0	0	0	0	1	1	1	6	6	48	14	2	4	100	4	0
10	2	23	17	35	23	32	15	10	0	0	0	0	3	5	3	8	10	100	32	15	7	83	3	2
11	1	23	4	64	13	15	1	10	0	0	0	1	1	2	1	7	2	24	65	33	2	72	3	1
12	1	16	6	29	10	33	6	5	0	0	0	0	1	2	1	10	3	41	11	2	3	34	1	1
13	1	22	5	60	12	14	1	10	0	0	0	2	1	2	1	7	2	23	60	31	2	70	7	1
14	1	12	3	39	7	12	3	4	0	0	1	0	1	1	1	6	1	22	7	2	2	20	1	0
15	2	23	5	59	12	27	4	8	0	0	0	1	1	2	1	9	3	36	32	23	5	100	4	1
16	1	15	3	32	20	40	5	8	0	0	0	0	1	1	1	5	10	73	12	5	3	100	3	1
17	0	20	4	55	14	39	4	10	0	0	0	0	0	2	2	14	4	51	4	4	2	43	2	0
18	0	13	4	32	19	9	1	9	0	0	0	0	0	1	0	4	1	41	16	19	1	51	1	0
19	0	22	5	50	25	58	5	12	0	0	8	10	0	0	0	5	5	80	5	7	3	70	3	0
20	2	18	5	39	13	11	5	9	0	2	0	0	1	1	0	2	1	18	2	1	5	77	2	1
21	1	15	3	17	8	13	2	9	0	0	0	0	0	1	1	11	4	34	3	2	1	40	1	0
22	1	20	5	69	18	62	5	12	0	0	0	0	0	1	1	6	6	82	16	7	2	64	2	1
23	0	18	5	61	21	60	4	10	1	1	2	2	0	1	0	6	5	100	10	10	2	61	2	1
24	0	17	4	62	16	57	4	9	0	0	0	0	0	1	0	6	5	100	14	9	2	54	2	1
25	0	15	4	77	16	57	3	9	0	0	0	0	0	0	0	6	6	91	10	15	1	48	1	0
26	0	11	3	69	14	50	4	8	0	0	0	0	1	1	1	5	6	86	10	16	2	40	1	1
27	0	8	2	56	10	44	10	6	0	0	0	0	0	1	1	4	5	77	7	14	2	32	1	1
28	0	3	1	28	6	43	2	1	0	0	0	0	0	0	0	2	2	27	6	21	2	8	1	0

Table 8. Continued

	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
1	0	1	0	0	0	0	0	34	33	10	1	96	7	1	3	12	66	4	0	0	0	1	0	1
2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	1	1	1
3	0	0	0	0	0	0	1	20	2	1	2	2	9	1	0	1	4	0	0	0	0	0	0	2
4	0	0	0	0	0	1	0	6	1	2	1	100	10	1	0	0	0	0	0	1	6	15	1	1
5	0	0	0	0	0	0	0	4	0	1	2	3	1	1	0	1	5	0	0	1	4	10	1	1
6	0	0	0	0	0	0	0	6	0	1	2	4	1	1	0	0	0	0	0	1	5	18	2	1
7	0	0	0	0	0	2	5	8	4	1	5	4	12	1	0	2	2	0	0	3	1	4	0	7
8	0	0	0	1	1	6	7	68	6	3	1	48	4	1	0	1	0	0	0	6	1	18	2	21
9	0	0	0	0	0	1	2	71	4	11	6	49	32	3	0	0	0	0	0	2	24	16	2	2
10	0	0	0	0	0	0	0	17	3	5	7	62	13	2	0	3	13	2	0	2	12	40	3	2
11	0	1	0	2	1	7	6	100	13	2	4	67	5	1	0	0	0	0	0	2	1	1	0	3
12	0	1	0	0	0	3	4	14	3	4	2	100	28	2	0	0	1	1	0	5	5	48	3	3
13	0	1	0	1	1	6	6	100	11	2	4	62	4	1	0	0	0	1	0	2	1	1	0	3
14	0	0	0	1	0	3	4	100	12	3	1	7	5	1	0	1	1	0	0	2	1	2	0	2
15	0	0	0	1	0	5	18	93	7	4	2	8	11	2	0	1	0	1	0	3	3	14	1	5
16	0	0	0	0	0	1	4	10	1	5	8	29	42	2	0	0	0	0	0	2	9	22	2	1
17	0	0	0	2	0	8	12	100	12	6	2	4	31	2	0	0	0	2	0	10	6	10	2	8
18	0	0	0	0	0	2	1	10	1	1	0	6	1	0	0	0	1	1	0	1	6	31	2	3
19	0	3	0	2	0	5	23	58	5	3	2	35	90	3	0	0	0	2	0	3	3	45	3	5
20	0	0	1	1	0	1	6	13	1	8	3	4	6	20	1	1	1	0	1	1	1	2	1	8
21	0	0	0	0	0	2	1	9	1	9	2	1	5	1	0	0	0	0	0	13	7	3	1	4
22	0	0	0	1	1	11	18	92	8	2	2	8	100	5	1	1	1	2	0	6	12	41	12	5
23	0	5	0	1	0	12	10	43	5	4	1	10	97	6	1	4	0	4	5	12	11	66	35	4
24	0	0	0	1	1	13	7	38	8	4	1	13	96	6	0	1	0	5	5	13	5	36	51	5
25	0	0	0	0	0	15	6	61	8	8	0	9	100	6	0	1	1	5	4	14	5	32	60	8
26	0	0	0	1	1	15	7	43	7	8	1	10	91	7	1	2	4	5	4	15	6	38	57	9
27	0	0	0	1	1	12	6	38	5	7	1	7	82	6	1	1	3	4	3	13	5	26	51	7
28	0	0	0	0	0	4	2	15	3	8	1	3	100	28	2	1	0	1	1	3	2	9	4	4

Table 8. Continued

	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	3	100	7	0	0	0	0	0	0	0
2	2	20	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
3	0	18	1	0	0	0	0	0	0	0	0	0	0	2	18	18	2	0	0	0	0	0	0	0
4	2	0	1	0	0	0	0	0	0	0	0	0	0	3	0	14	0	0	0	0	0	0	0	0
5	0	14	67	3	0	0	0	0	0	0	0	0	0	5	0	2	0	0	0	0	0	0	0	0
6	1	15	61	4	0	0	0	0	0	0	0	0	0	9	1	5	1	0	0	0	0	0	0	0
7	0	25	3	1	0	1	1	1	1	3	1	12	1	2	78	48	5	0	0	0	0	0	0	1
8	1	5	1	0	0	0	0	0	0	5	7	25	8	4	31	4	1	0	0	0	0	0	0	0
9	9	2	3	0	0	0	0	0	0	2	0	0	0	10	3	67	4	0	0	0	0	0	0	1
10	3	17	37	3	0	0	0	0	0	0	0	0	0	32	3	17	2	0	0	0	0	0	0	0
11	2	12	75	4	1	0	0	0	0	2	4	15	2	2	1	53	7	1	0	0	0	0	0	0
12	2	7	1	2	0	0	0	0	0	1	5	7	1	2	1	1	0	0	0	0	0	0	0	7
13	2	12	78	4	0	0	0	0	0	2	5	15	3	3	1	49	7	1	0	0	0	0	0	0
14	1	15	2	0	0	0	0	0	0	1	4	56	5	4	1	2	24	2	0	0	0	0	0	0
15	1	38	79	4	0	0	0	0	0	2	16	29	2	3	2	52	5	0	0	0	0	0	0	1
16	2	13	4	0	0	0	0	0	0	0	0	4	0	2	3	54	4	0	0	0	0	0	0	0
17	2	51	4	2	0	0	0	0	0	4	12	69	6	2	0	2	0	0	0	0	0	0	0	14
18	0	8	100	4	0	0	0	0	0	1	0	0	0	0	2	7	1	0	0	0	0	0	0	0
19	0	23	3	0	0	0	0	0	0	2	10	27	3	2	13	5	2	0	0	0	0	0	0	0
20	1	2	4	0	0	0	0	0	0	23	3	1	7	38	11	20	1	1	0	0	0	0	0	0
21	2	1	3	4	0	0	0	0	0	1	0	2	1	10	1	1	0	0	0	0	0	0	1	25
22	0	42	5	0	0	1	1	2	1	2	4	71	5	2	1	9	0	0	0	0	0	0	0	2
23	0	35	6	1	0	1	2	2	0	5	12	27	20	2	2	11	1	0	0	1	1	4	1	2
24	0	36	4	0	0	1	0	3	4	8	10	45	68	5	1	10	1	0	1	1	1	5	0	3
25	0	37	4	0	0	1	0	6	3	8	8	23	100	8	1	6	0	0	0	0	0	3	3	5
26	0	37	5	0	0	1	1	5	4	10	9	26	100	10	1	7	1	0	0	1	0	4	2	5
27	0	31	4	0	0	1	0	3	3	10	8	24	100	9	1	5	1	0	0	1	1	3	3	5
28	0	71	6	1	0	1	0	1	0	2	1	7	3	1	1	5	1	0	0	0	0	1	0	1

Table 8. Continued

	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133
1	0	0	0	1	0	7	15	1	0	0	0	0	0	0	0	0	0	0	4	30	1	0	0	0
2	0	0	0	0	27	100	7	1																
3	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	27	43	3	1		
4	0	0	0	0	20	24	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
5	0	0	0	1	18	19	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
6	0	0	0	1	30	34	4	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0
7	1	1	2	0	3	3	0	0	0	0	0	0	0	1	0	0	0	0	45	68	6			
8	0	0	0	37	3	8	1	0	0	0	0	0	0	0	0	0	1	23	69	87	8	1	1	1
9	0	1	1	3	67	30	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
10	2	0	0	2	90	47	5	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0
11	0	1	0	44	22	2	0	0	0	0	0	0	0	0	1	0	1	2	99	61	6	1	0	0
12	0	0	1	10	7	52	4	1	0	0	0	0	0	0	0	0	0	0	29	9	1	1	0	0
13	0	1	0	46	18	2	0	0	0	0	0	0	0	0	1	0	1	2	94	59	6	0	0	0
14	0	0	0	4	1	7	1	0	0	0	0	0	0	0	0	0	0	0	9	49	4	1	5	0
15	0	0	1	27	12	25	2	0	0	0	0	0	0	0	0	0	0	1	52	25	2	0	1	0
16	3	46	4	2	58	16	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
17	4	4	2	18	2	0	0	0	0	0	0	0	0	0	0	0	0	6	16	100	8	2	0	0
18	1	9	1	0	0	26	1	0	0	0	0	0	0	0	0	0	0	2	6	1	0	0	0	0
19	0	13	2	5	8	100	7	0	0	0	0	0	0	2	20	33	3	2	32	7	0	0	0	0
20	0	0	0	21	3	12	1	7	1	0	0	0	0	0	0	1	59	100	9	1	0	0	0	1
21	2	1	10	38	6	25	2	0	0	0	0	0	0	0	0	1	1	33	3	9	1	2	0	0
22	19	25	3	4	12	3	2	0	0	0	0	1	0	0	0	1	0	3	5	91	7	1	0	0
23	8	50	5	4	2	4	1	0	0	0	0	0	0	4	24	21	2	1	6	1	5	0	0	0
24	6	9	6	2	3	9	1	0	0	1	2	9	2	2	8	57	6	2	1	2	1	0	0	0
25	5	14	32	3	1	4	1	0	0	0	0	1	1	3	3	4	4	1	1	3	1	0	0	0
26	4	11	31	4	1	4	1	0	0	1	1	4	2	4	4	10	13	1	1	3	1	1	0	1
27	4	10	27	3	1	4	1	0	0	1	1	5	1	3	2	5	7	1	1	3	2	1	0	1
28	1	3	1	1	0	2	1	0	0	0	0	1	0	1	0	1	0	0	0	6	2	0	0	0

Table 8. Continued

	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2																								
3																								
4	0	0	0	0	0	0	0	0	13	14	1													
5	0	0	0	0	0	0	0	0	6	23	2													
6	0	0	0	0	0	0	0	0	13	40	5	1												
7																								
8	0	0	0	0	0	0	0	2	0	1	0	49	3	1	0	0	0	0	0	0	0	0	5	30
9	0	0	0	0	0	0	0	1	24	54	5	1												
10	0	0	0	0	0	0	0	0	23	55	5	2												
11	0	0	0	0	0	0	0	1	1	2	0	0	3	0	0	0	0	0	0	0	0	0	25	61
12	0	0	0	0	0	0	0	17	2	1	0	0	0	0	0	0	0	0	0	0	0	0	13	29
13	0	0	0	0	0	0	0	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	24	58
14	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	8
15	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	38
16	0	0	0	0	0	0	0	0	11	40	4													
17	0	0	0	0	0	0	2	49	16	6	0	0	0	0	0	0	0	0	0	0	0	4	0	0
18	0	0	0	0	0	1	0	0	14	11	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	42
20	0	0	0	0	0	1	0	1	0	0	0	7	1	0	0	0	0	0	0	1	20	8	1	1
21	0	0	0	0	0	0	20	100	10	2	0	0	0	0	0	0	0	0	0	2	0	0	1	1
22	0	0	0	3	76	18	2	0	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	1	2	0	4	0	0	2	4	4	45	5	0	0	0	0	0	0	2	82	12	1	0	6	2
24	0	0	0	3	33	16	2	1	2	1	5	0	0	0	5	2	0	1	0	0	1	0	2	45
25	3	13	1	1	3	1	0	0	1	4	0	0	0	1	1	4	1	1	6	25	3	0	0	0
26	1	2	1	2	3	3	3	1	1	3	1	1	0	1	2	15	2	1	2	1	1	0	2	2
27	1	2	1	3	3	5	8	1	0	2	1	0	0	1	1	1	1	1	2	1	3	1	1	1
28	0	1	0	0	0	1	0	0	0	14	2	0	0	0	0	1	0	0	0	0	0	0	0	2

Table 8. Continued

	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181
1	0	0	6																					
2																								
3																								
4																								
5																								
6																								
7																								
8	3	6	72	8	1	0	0	0	0	0	0	0	0	0	0	1								
9																								
10																								
11	7	1																						
12	3																							
13	7	1																						
14	1																							
15	4																							
16																								
17	0	0	0	0	0	0	0	0	0	0	0	0	8	36	4	2								
18	0	0	0	0	0	0	0	0	0	0	0	0	1	9	1									
19	3																							
20	9	13	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
21	0	17	2	0	0	0	0	0	0	0	1	0	0	0	0	5	6	1	0	0	0	0	0	0
22	0	0	1	0	0	0	0	0	0	0	0	0	6	56	6	1								
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	4	0	0	0	0	0	0	0	30	4	0	0	4	1	0	0	0	0	0	0	0	0	1	0
25	0	0	0	0	0	0	0	0	0	5	3	0	0	3	0	0	0	0	0	0	0	0	0	0
26	1	0	0	0	1	3	1	1	5	19	2	0	0	1	0	1	0	0	0	0	0	1	4	2
27	1	0	0	0	1	9	1	1	1	1	1	1	1	2	0	0	0	0	0	2	1	1	4	13
28	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Table 8. Continued

	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205
20	0	0	0	0	46	80	9	1																
21	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	32	4	1		
22																								
23	0	0	7	44	6	1																		
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	44	6					
25	0	0	1	23	3	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0
26	0	0	3	1	2	0	0	0	1	1	0	0	0	0	0	0	1	23	3	0	0	0	0	0
27	2	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0	1	1	1	0	0	0	0	1
28	0	0	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	5	1	0	0	0	0	0

	206	207	208	209	210	211	212	213	214	...	221	222	223	224	225	226	227	228	...	240	241	242	...	254
25	0	1	0	0	0	0	0	0	0		0	0	0	0	0	1	19	3						
26	0	3	6	2	0	0	1	0	0		0	0	0	0	0	0	0	0		1	20	3		
27	0	2	1	0	0	0	1	18	3		1	5	1	0	0	0	0	0		0	0	0		1
28	0	0	0	0	0	0	0	2	1		0	0	0	1	0	0	1	0		0	1	1		0

	255	256	...	267	268	269	...	298	299	300
27	17	3								
28	7	1		4	1	1		16	4	1

yielding 4.0 g. (0.021 mole, 100%) of a clear, colorless liquid.

Dimethyl 2,5-Dimethyladipate

This methyl ester was purchased (Columbia D6120) and used as received.

Dimethyl Tridecanedioate

This compound (Columbia T2615) was used as received.

meso-2,3-Dimethylsuccinic Acid

A 1.26-g. (0.010 mole) sample of 2,3-dimethylmaleic anhydride (columbia D6505) was hydrogenated at room temperature and atmospheric pressure in acetic acid using 1.00 g. of five per cent platinium on carbon. At the end of five hours the uptake of hydrogen had ceased (230 ml., 92%). Water was added to the mixture; it was boiled under reflux for ca. 0.5 hr. The mixture was filtered through a celite bed and the filtrate was evaporated. The solid that was obtained was recrystallized from water; this yielded 1.05 g. (0.072 mole, 72%) of product.

Comments on Background Mass Spectra

In all mass spectral investigations, the background inherent to the particular system being used must be considered. The term "background mass spectra" means the actual mass spectra that are obtained when no component is purposely introduced into the ionization chamber. When using the combined techniques of GLC-MS, particularly intense background may be encountered because of the continual "bleed" of the liquid phase from the GLC column. Many background spectra were determined during the course of this investigation. They were determined at different temperatures and with different columns (all SE-30) that had been used for various lengths of time. It was found that the background spectra

varied (both in relative and absolute intensities) with temperature and with the history of the column. The absolute intensities of background peaks increased with both increasing temperature and increasing time that the column was used. In general, peaks were observed in the background spectra (in approximate order of decreasing intensity) at m/e 28 (N_2), 32 (O_2), 40 (A), 207,208,209,73,55,57,44,96,97,133,135,157,191, 193,221,222,223,281,282,283,355,356,357,295,296,297,215,265,267,268,269, 315,325,327,339,341,342,343,369,370,371,401,415,416, and 417. In this list, groups of peaks are always listed together (i.e., in the group 207, 208, and 209, the peak at m/e 209 may be less intense than peak listed after it).

In the low temperature runs, the most intense background peaks (besides O_2, N_2, and A) were approximately 1-2% of the base peak. In the high temperature runs, they were approximately 5% of the base peak.

Preparation of p-Phenylphenacyl Esters

The pH of a solution of approximately five millimoles of the acid in three milliliters of water was adjusted to 5 using solid potassium carbonate. To this solution was added an equivalent amount of p-phenylphenacyl bromide and ten milliliters of absolute ethanol. The reaction mixture was boiled under reflux for ca. two hours and was then allowed to cool to room temperature. The solid was collected, washed with water and ice cold 95% ethanol. The esters were recrystallized from ethanol-water; their melting points are summarized in Table 9.

Table 9. p-Phenylphenacyl Esters Prepared for GLC Standards

Acid Used	m.p. of Product	Lit. m.p.	Ref.
Acetic	110-112°	110.5-111°	47
Propionic	101-102°	103-103.5°	47
Isobutyric	87-90°	88.5°	48
n-Butyric	82.5-84°	82-83°	47

Nitric Acid Oxidations of Flavensomycin, Perhydro Fragment, and Perhydroflavensomycin

General Procedure

The weighed sample that was to be oxidized was placed in a 100-ml. three-necked, round-bottomed flask. This flask was equipped with a gas inlet tube* and a reflux condenser (ice water was circulated in the condenser). Twenty milliliters of concentrated nitric acid was added to the flask, and the contents were cooled to 0° (ice-water bath). The temperature of the reaction mixture was then varied: one hour at 0°, one hour at room temperature, one hour at ca. 50°, and finally, two hours at steam bath temperature.

Flavensomycin

Determination of Carbon Dioxide. When 99.0 mg. (0.114 mmole) of flavensomycin was used, 305.6 mg. (1.31 mmole) of barium sulfate was

* The gas inlet tube was used in conjunction with the determination of carbon dioxide. For the details of this determination see p. 37, this thesis. In cases where carbon dioxide was not determined, this was not used.

isolated. This corresponded to 11.5 moles of carbon dioxide from one mole of flavensomycin. In another oxidation, 58.2 mg. (0.0667 mmole) of flavensomycin was used; 232.6 mg. (0.998 mmole) of barium sulfate was isolated, corresponding to 14.9 moles of carbon dioxide from one mole of flavensomycin.

Determination of Volatile Acids. The aqueous portion from the nitric acid oxidation of 58.2 mg. (0.0667 mmole) of flavensomycin was neutralized to pH ca. 1.5 with concentrated sodium hydroxide solution. This solution (ca. 50 ml.) was distilled to dryness under aspirator vacuum; the receiving flask was cooled in an ice bath. An additional 50 ml. of water was added, and the process was repeated. The total distillate was made basic (ca. pH 11) with sodium hydroxide solution, and then was lyopholyzed to dryness. The solid was dissolved in two milliliters of water, and the pH was adjusted to 5. To this solution was added 130 mg. of p-phenylphenacyl bromide and 10 ml. of ethanol. The mixture was boiled under reflux for two hours, diluted with ca. 50 ml. of water, and extracted several times with chloroform. The chloroform solution was dried and evaporated, yielding 119.2 mg. of a yellow crystalline solid. GLC (6-ft. column, 3% SE-30 CT 196°, AIP 20 psi) showed, among others, peaks at R.T. 7.4 and 11.7 min., relative areas ca. 14:1. Under the same conditions the p-phenylphenacyl esters of acetic and iso-butyric acids showed R.T. 7.2 and 11.6 min., respectively. A very small peak was observed at R.T. 10.0 min. (relative area ca. 1-2% of the peak at 7.4 min.) which corresponded to the p-phenylphenacyl ester of propionic acid (R.T. 9.9 min.). No other peak beyond the one at R.T. 11.7 min. was observed (the recorder was run long enough for the p-phenylphenacyl ester of hexanoic acid

to have been eluted).

Analysis of the Methyl Esters of the Nonvolatile Acids by GLC-MS. A 200-mg. portion of flavensomycin was oxidized with concentrated nitric acid in the usual manner. The solution was neutralized to pH ca. 1 with sodium hydroxide solution and continuously extracted with chloroform for three days. The chloroform was dried and evaporated, yielding 40 mg. of yellow oil. This material was dissolved in methanol and reacted with a large excess of ethereal diazomethane. After standing overnight, the solvents were evaporated, and dry benzene was distilled from the residue several times. The results of combined GLC-MS analysis are summarized in Tables 10 and 11. In another oxidation, 121.0 mg. of flavensomycin yielded 29.2 mg. of yellow oil. This oil was converted to methyl esters by the dimethoxypropane method.* The results of GLC analysis of this preparation are summarized in Tables 12 and 13.

Perhydro Fragment

Determination of Carbon Dioxide. When 1.23 g. of the perhydro fragment (from the catalytic reduction of flavensomycin in acetic acid, chromatographed on acid-washed alumina) was oxidized with concentrated nitric acid, 1.4282 g. (0.00613 mole) of barium sulfate was isolated. If the molecular weight of this material was ca. 673 (872 + 14 - 213) then this would correspond to about 3.3 moles of carbon dioxide per mole of perhydro fragment.

Determination of Volatile Acids. The pH of the aqueous portion from the above oxidation (ca. 200 ml.) was adjusted to about 1.0 with

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* See p. 39, this thesis.

Table 10. Mass Spectral Data from Nitric Acid Oxidation of Flavensomycin. Low Temperature.*

Components in Order of Elution**	Relative Intensities of Peaks at m/e =																						
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	118	88	35	37	51	5	0	0	1	4	8	27	5	5	7	89	20	100	4	1	0	3	21	31
2	111	off	34	77	43	12	0	0	1	3	4	12	5	7	6	52	20	100	3	1	0	2	14	15
3	118	79	off	30	46	4	0	0	1	10	24	100	73	9	5	31	16	39	37	1	0	2	10	15
4	29	18	5	12	4	6	0	0	0	0	1	3	1	7	12	off	8	18	1	2	0	0	1	1
5	92	61	72	39	38	35	1	0	0	2	7	31	13	36	16	44	11	25	6	2	0	1	5	7
6	86	75	15	59	37	30	1	0	0	3	6	31	12	13	14	off	23	100	3	4	0	1	5	6
7	91	89	16	62	27	18	1	0	0	2	3	10	4	18	25	67	15	100	4	3	0	0	3	4
8	72	52	8	48	33	13	0	1	0	2	4	20	5	30	26	74	13	76	2	2	0	2	3	4
9	62	75	12	44	27	34	1	0	0	2	3	17	5	18	9	41	10	52	2	2	0	1	3	3
10	61	61	11	40	28	18	0	0	0	1	2	11	3	14	40	65	11	64	2	2	0	1	2	3
11	78	66	34	40	29	14	0	0	0	1	3	13	4	12	11	91	14	100	3	5	0	1	3	4

* Six foot column, 3% SE-30, RF = 40 cc./min., operated at 40° initially and programmed to ca. 100° at about 3°/min.

** The first peak to be eluted from the column after solvent was labeled 1, and mass spectra were taken of it and the next ten peaks.

Table 10. Continued.

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	28	4	2	3	3	3	3	off	12	9	3	7	1	4	0	0	0	1	4	1	0	4	7	3
2	16	1	1	2	10	60	4	off	45	19	2	3	0	0	0	0	0	1	2	0	1	6	4	2
3	15	3	2	41	19	13	3	89	11	5	1	2	0	0	0	0	2	1	3	1	1	5	5	2
4	1	2	2	92	11	10	3	79	2	3	0	0	0	1	0	0	1	1	1	1	2	2	2	9
5	5	5	2	33	10	24	8	100	5	6	1	1	0	2	1	2	2	4	70	5	6	8	8	3
6	6	7	2	12	5	8	5	78	5	7	2	1	0	1	1	5	10	9	3	3	19	14	3	14
7	4	4	4	off	44	45	19	97	13	5	2	1	0	1	1	2	3	6	2	3	17	36	9	8
8	4	2	2	33	5	9	8	100	8	4	2	2	1	4	1	2	2	8	4	5	17	16	17	8
9	3	3	2	19	22	88	15	55	5	40	3	1	0	1	1	2	3	7	2	10	11	10	3	5
10	3	3	3	22	13	33	10	100	10	16	1	1	1	1	1	2	2	6	3	17	8	19	11	5
11	4	3	2	11	11	34	8	off	11	47	2	1	0	1	1	19	2	6	2	9	4	11	11	9

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
1	7	26	99	12	0	0	0	0	0	0	0	0	2	2	2	77	40	4	0	0	2	0	0	0
2	4	16	73	6	0	0	0	0	0	0	0	7	2	0	0	0	0	0	0	0	2	0	0	0
3	2	10	53	4	0	0	0	0	0	0	0	2	2	0	2	2	0	0	0	0	1	0	0	0
4	1	1	3	1	0	0	0	2	1	2	3	23	2	1	0	1	0	0	0	0	0	0	0	1
5	2	9	15	2	0	1	1	4	1	22	2	15	22	11	2	3	0	1	0	1	1	1	1	2
6	3	8	19	2	0	1	1	2	1	5	1	3	3	13	2	2	0	0	0	1	2	1	3	18
7	1	4	14	2	0	1	1	4	4	6	4	43	42	9	2	1	0	0	0	1	1	2	1	4
8	2	5	16	2	0	1	2	2	2	18	2	19	4	5	5	2	0	2	0	1	2	5	2	5
9	1	3	11	1	0	1	1	3	2	100	7	10	27	5	4	1	1	1	1	1	1	1	2	7
10	1	3	10	2	0	1	2	4	1	41	3	11	18	11	3	2	2	1	0	1	1	1	1	5
11	1	4	11	1	0	1	1	1	1	41	3	5	9	6	1	1	1	0	0	0	1	1	1	4

Table 10. Continued

	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
1	0	0	3	3	1	9	19	2	0	0	0	0	0	0	0	0	0	0	9	2	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0
4	0	2	2	29	2	1	0	0	0	0	0	0	0	1	32	100	7	1	0	1				
5	14	15	2	2	3	1	0	0	1	1	1	1	1	2	4	12	12	16	1	2	1	0	0	3
6	3	7	2	23	13	7	1	0	0	0	1	1	1	2	3	7	1	18	1	1	1	0	0	0
7	16	99	8	6	4	1	0	0	0	0	1	6	1	4	27	off	17	5	1	1	0	0	0	0
8	32	27	7	5	2	1	0	0	2	0	0	1	1	2	3	10	1	11	1	1	0	0	0	0
9	3	4	1	9	3	1	0	0	0	0	2	1	2	4	2	5	7	95	8	1	0	0	0	0
10	3	17	3	12	2	1	0	0	1	1	1	2	1	2	5	6	3	75	6	2	1	1	0	0
11	1	3	4	9	1	27	1	1	0	1	0	1	1	4	1	4	4	43	3	2	1	1	0	0

	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
1	0	0	0	0	0	0	0	0	2	2														
2	0	0	0	0	0	0	0	0	0	2														
3	0	0	0	0	0	0	0	0	1	2														
4																								
5	0	0	0	1	16	25	2																	
6	0	0	7	73	7	2	0	0	0	2	0	0	2	0	0	0	0	0	0	2	0	0	0	0
7	0	0	1	4	26	42	3	1	2	11	1	0	0	0	0	0	0	0	0	0	0	0	0	0
8	2	0	0	2	17	34	2	5	1	1	0	0	0	0	0	2	0	0	3	3	0	0	0	0
9	0	0	1	10	3	2	1	1	0	1	0	0	0	0	0	0	2	1	0	1	2	4	1	
10	0	0	1	2	3	9	2	4	1	1	0	0	0	0	0	0	1	1	2	4	2	2	1	4
11	0	0	0	2	4	2	4	5	0	off	29	5	0	1	0	0	0	1	0	0	1	2	0	0

Table 10. Continued

	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171
1																								
2																								
3																								
4																								
5																								
6	0	0	0	0	0	0	0	0	0	0	2													
7	0	0	0	0	0	0	0	0	0	0	0	0	2											
8	0	0	0	0	2	1	0	2																
9																								
10	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	3	0	0	14	2	1								

	172	173	174
1			
2			
3			
4			
5			
6			
7			
8			
9			
10	0	0	1
11			

Table 11. Mass Spectral Data from Nitric Acid Oxidation of Flavensomycin. High Temperature.*

Components in Order of Elution**	Relative Intensities of Peaks at m/e =																							
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	23	36	5	19	6	3	0	0	0	2	3	24	6	61	16	100	10	17	1	1	0	0	4	6
4	68	60	12	25	27	6	0	0	0	3	6	45	12	100	30	off	28	31	2	2	0	1	10	15
5	124	90	51	87	42	12	0	0	0	6	8	53	16	79	30	off	55	100	6	7	0	2	14	22
6	73	41	11	21	29	4	0	0	0	3	5	32	8	56	18	100	28	21	1	2	0	1	8	13
7	53	75	7	16	23	4	0	0	1	2	4	33	9	100	31	off	21	19	1	2	0	0	5	8
10	96	29	9	17	35	3	0	0	1	3	4	29	7	49	14	100	28	17	1	2	0	1	7	13
13	110	35	6	13	52	2	0	0	1	1	3	21	6	58	13	100	30	13	1	1	0	1	5	8
20	85	67	8	16	34	3	0	0	1	1	2	23	6	67	19	100	28	79	2	2	0	1	6	8
22	72	49	7	13	25	2	0	0	1	1	2	22	5	83	19	100	22	15	1	1	0	1	5	6

* Six-foot column, 3% SE-30, RF = 40 cc./min., initial temperature 170°, isothermal for ca. 26 min. and then programmed at about 5°/min. to 260°.

** In total, 24 mass spectra were taken; these represent only the most intense peaks.

Table 11. Continued

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	6	11	5	53	12	15	5	55	3	2	1	2	1	4	3	16	7	42	5	7	3	9	53	8
4	12	21	12	83	21	29	9	86	6	5	4	9	3	9	6	31	14	75	12	17	6	17	90	15
5	22	28	12	51	24	28	16	100	9	41	2	6	3	15	9	39	15	59	11	19	8	18	19	51
6	10	17	7	39	13	18	8	54	6	7	4	9	3	9	5	13	8	36	9	12	4	11	15	9
7	7	17	12	87	23	53	7	60	3	4	1	3	1	6	5	24	10	60	11	24	8	14	off	62
10	9	16	6	31	11	18	5	55	3	3	3	7	3	11	6	16	7	30	5	10	4	8	20	10
13	6	12	6	43	10	28	4	37	2	2	0	3	1	5	3	14	7	30	6	8	3	9	82	22
20	6	13	6	45	72	96	8	44	3	4	1	7	2	11	6	17	7	30	7	20	6	21	10	11
22	5	13	7	60	25	86	7	32	2	2	1	2	1	10	4	16	8	35	46	63	5	23	6	8

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
1	2	7	16	7	3	12	6	17	26	10	1	22	8	2	1	3	1	4	3	12	7	19	39	9
4	5	20	35	16	7	23	12	43	55	20	3	40	11	10	7	11	3	11	7	23	14	30	84	18
5	7	26	50	16	7	20	15	32	10	24	5	12	16	52	6	24	4	15	8	30	16	24	11	20
6	4	18	29	11	4	12	9	20	13	13	3	11	16	12	6	9	3	7	4	14	9	15	20	11
7	6	11	20	9	4	16	8	37	21	16	2	off	21	4	1	5	2	7	4	15	8	25	26	14
10	4	20	21	13	4	11	7	17	6	10	2	15	5	7	3	17	3	7	3	12	6	12	9	7
13	3	11	17	9	3	10	7	22	7	11	2	52	7	3	2	8	2	6	3	10	6	16	6	7
20	10	15	9	11	4	13	8	26	7	48	4	9	6	4	2	13	3	10	4	15	8	15	6	10
22	8	15	7	9	4	13	9	34	13	13	2	5	3	4	2	10	2	9	4	13	7	13	5	8

Table 11. Continued

	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
1	3	7	3	2	2	3	1	3	3	10	5	10	13	9	3	5	1	3	1	1	2	7	3	5
4	5	14	4	6	4	11	3	9	5	16	9	24	27	15	7	11	4	8	4	6	3	9	7	13
5	7	20	6	8	10	35	6	9	8	25	11	29	13	20	8	12	5	9	5	17	6	31	11	24
6	3	10	4	4	4	9	3	6	4	12	8	16	11	11	4	6	4	8	4	6	3	7	5	10
7	3	20	3	3	3	6	2	6	4	11	5	17	11	8	3	10	4	4	1	4	2	6	5	9
10	3	9	5	10	5	11	2	7	4	10	4	12	6	8	3	19	8	18	13	8	3	7	5	9
13	3	7	2	3	2	7	2	4	3	8	4	12	4	5	2	5	2	4	1	4	2	6	4	7
20	20	37	6	6	18	17	4	6	4	12	7	12	5	9	3	9	4	10	4	8	3	9	7	10
22	2	4	2	5	14	13	2	5	4	10	5	10	17	25	3	7	2	5	2	5	2	8	7	10

	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
1	3	6	3	9	4	5	1	1	0	2	2	4	2	7	5	21	3	16	3	3	8	3	1	1
4	8	13	9	20	7	9	4	4	3	9	5	17	5	8	7	9	5	11	5	9	3	4	2	4
5	10	20	12	33	11	12	5	5	3	8	5	20	7	21	8	15	9	16	6	11	6	4	3	5
6	5	10	6	13	5	6	2	3	3	7	5	7	4	8	4	7	4	11	4	5	3	4	2	5
7	4	11	7	9	4	19	5	2	2	4	2	4	3	6	3	7	4	6	2	35	5	2	1	2
10	4	12	6	10	5	9	3	6	6	9	3	5	3	6	5	7	4	7	3	7	3	11	7	5
13	2	7	4	7	4	7	2	2	1	4	2	5	2	5	2	5	2	4	2	12	2	2	2	3
20	5	9	5	10	6	7	2	5	9	14	4	8	3	6	3	7	4	7	3	6	2	4	3	10
22	4	7	4	7	4	4	2	4	5	8	2	8	2	5	3	5	3	5	2	5	2	4	3	10

Table 11. Continued

	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171
1	1	1	1	3	9	14	3	7	2	2	1	2	0	1	1	1	2	2	5	4	3	5	2	14
4	4	8	4	8	9	37	8	12	4	6	2	3	2	4	2	3	2	5	8	9	4	7	5	5
5	5	33	9	16	7	33	11	20	7	7	2	5	2	5	3	6	6	18	7	11	6	11	4	9
6	3	7	4	7	5	20	6	8	3	4	2	3	2	3	2	3	3	5	5	7	4	7	3	4
7	2	11	3	7	3	11	8	7	2	6	2	2	2	2	2	3	2	4	4	9	4	4	2	9
10	3	4	2	5	3	13	5	7	2	3	1	5	3	5	3	4	2	5	5	7	3	5	2	4
13	2	5	2	4	3	10	3	4	2	3	1	2	1	3	2	3	2	4	2	7	2	3	2	3
20	7	45	7	6	4	10	4	8	3	5	2	4	3	5	10	6	3	4	3	6	3	4	2	4
22	4	off	21	5	4	9	3	5	2	4	2	3	2	4	3	4	2	4	3	60	7	4	2	3

	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	182	193	194	195
1	2	3	0	0	0	1	1	2	3	3	2	2	2	3	1	3	0	0	0	0	0	1	1	1
4	2	2	1	15	6	4	2	5	3	5	3	10	5	27	4	3	1	2	1	4	3	3	8	4
5	4	3	2	2	2	13	5	11	6	10	5	14	6	13	4	3	2	2	2	4	4	5	4	6
6	2	3	1	9	5	5	3	5	3	4	3	6	4	5	2	2	2	2	1	3	2	4	4	4
7	2	2	2	2	2	3	2	4	3	4	2	5	4	8	3	1	1	1	1	2	2	3	2	3
10	1	3	2	6	9	6	2	5	3	6	2	6	2	3	2	3	1	7	2	6	2	3	3	3
13	2	2	2	2	2	5	3	5	3	5	2	4	2	4	1	1	1	2	0	2	0	3	2	4
20	2	4	4	4	13	6	4	4	4	4	2	4	3	4	2	3	2	4	3	6	2	17	5	4
22	2	3	2	3	2	4	2	4	3	4	2	3	2	3	1	3	2	4	3	4	2	4	2	3

Table 11. Continued

	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219
1	1	4	3	3	1	3	1	0	0	0	0	1	1	1	2	24	4	11	3	2	0	0	0	0
4	2	7	4	5	1	2	1	1	1	2	1	5	2	3	3	6	2	5	1	3	1	1	1	18
5	5	47	10	7	2	3	2	2	2	3	2	8	4	20	6	12	4	7	2	5	2	2	1	2
6	2	5	3	6	2	2	1	3	3	5	3	6	3	4	3	6	3	4	2	3	1	1	1	4
7	2	4	2	12	2	2	1	1	1	2	1	4	3	4	2	4	2	4	2	2	1	1	1	1
10	2	5	3	4	1	2	2	44	19	8	4	9	3	3	2	5	2	3	1	2	1	2	1	6
13	0	3	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	2	7	3	7	2	3	2	4	7	6	2	12	4	4	2	4	2	4	2	3	2	3	4	4
22	2	4	2	4	2	2	2	4	5	4	2	11	4	4	2	2	2	4	2	2	2	2	5	4

	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243
1	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	3
4	4	2	2	2	1	4	5	22	4	4	1	0	0	0	0	37	7	2	2	2	1	3	1	7
5	2	2	2	3	3	8	3	7	2	6	2	2	1	1	2	2	2	3	2	3	2	5	2	7
6	2	3	2	2	2	4	2	7	2	3	1	1	1	1	5	54	9	2	2	3	2	4	0	0
7	2	2	1	2	2	3	2	15	3	2	0	1	1	1	1	2	1	1	1	9	3	11	2	2
10	3	3	3	3	2	3	1	2	1	1	1	2	1	3	4	52	9	3	2	1	1	5	1	2
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
20	3	7	2	3	2	2	2	3	2	5	2	2	2	6	2	4	2	3	2	2	1	2	1	2
22	2	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9

Table 11. Continued

	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267
1	0	0	0	0	0	0	0	0	0	0	0	0	0	1										
4	1	1	0	0	0	0	12	7	2	1	1	1	1	2	1	1	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	19	6	2	2	2	0	0	0	0	0	0
6	0	0	0	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	2
7	1	1	1	1	1	1	1	1	2	2	1	2	2	2	1	1	0	0	0	0	0	2	1	2
10	0	1	1	3	1	2	2	2	1	2	1	3	1	2	1	1	1	2	73	47	9	2	1	1
13	0	0	0	0	0	0	0	8	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
20	2	2	2	2	5	7	4	3	2	2	1	2	1	6	2	2	1	2	2	3	2	3	1	2
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291
1																								
4	0	0	0	0	0	4	1	1																
5	4	2	1	7																				
6	0	0	0	3																				
7	1	1	21	5																				
10	1	2	1	3	1	1	1	1	1	1	2	3	1	2	1	1	2	2	1	1	1	2	1	0
13	0	3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
20	1	2	1	1	1	2	1	2	2	2	2	3	2	6										
22	0	0	0	0	0	0	0	0	0	0	0	14	4	6										

Table 11. Continued

	292	293	294	295	296	297	298	299	300	301
1										
4										
5										
6										
7										
10	1	1	10	2	1	1	1	1	1	2
13	0	0	0	0	0	0	0	9		
20										
22										

Table 12. GLC Data from Nitric Acid Oxidation of Flavensomycin. Low Temperature.*

Retention Times, in Min.	Relative Area	Possible Identification Based Solely on Retention time (as Dimethyl Esters).
8.17	2	
8.45	1	
8.70	2	
9.44	100	
9.61	34	Succinic (9.6, 9.6)**
10.6	7	2-Methylsuccinic (10.6, 10.6)
11.4	10	2,2-Dimethylsuccinic (11.5, 11.6)
11.95	14	
13.4	3	Glutaric (13.2, 13.3)
14.1	2	
14.68	5	
15.1	13	3-Methylglutaric (15.1, 15.1)
16.18	15	2,4-Dimethylglutaric (lower boiling) (16.1, 16.1)
19.1	4	2,3-Dimethylglutaric (19.3, 19.3)
19.7	6	Adipic (19.6, 19.7)
20.52	5	
21.75	2	
30.0	4	Pimelic (31)***
40.0	2	
47.05	9	

* Thirty-foot column, 3% SE-30, FR 30 cc./min., CT 136°; all retention times are from injection; standards were run immediately before and after injection for comparison of retention times.

** The values given in parentheses are the retention times of those standards.

*** Retention time estimated from a semi-logarithmic plot of other straight chain standards.

Table 13. GLC Data from Nitric Acid Oxidation of Flavensomycin. High Temperature.*

Retention Times, in Min.	Relative Area	Possible Identification Based Solely on Retention Time (as Methyl Ester).
2.75	40	Pimelic (2.7, 2.7)**
3.3	100	Suberic (3.3, 3.3)
3.7	1	
3.9	17	
4.2	37	Azelaic (4.2, 4.2)
4.75	6	
5.0	6	
5.45	10	Sebacic (5.4, 5.4)
6.4	1	
6.6	2	
7.2	9	Undecanedioic (7.1)***
8.0	1	
8.3	1	
8.8	3	
9.65	4	Dodecanedioic (9.6, 9.8)
10.75	2	
11.8	2	
12.8	7	Hexadecanoic (12.7, 12.9)
13.1	5	Tridecanedioic (13.1)***
18.15	4	Tetradecanedioic (18.1, 18.3)

* Twelve-foot column, 3% SE-30, FR 30 cc./min., CT 195°. All retention times are from injection. Standards were run immediately before and after injection for comparison of retention times.

** The values given in parentheses are the retention times of those standards.

*** Retention time estimated from a semi-logarithmic plot of other straight chain standards.

solid sodium carbonate. The solution was then distilled to dryness, 50 ml. of water was added, and the solution was distilled to dryness again (the receiving flask was cooled in an ice bath). The distillate was made basic with sodium hydroxide solution and evaporated. Part of this material (40%) was converted to p-phenylphenacyl esters in the standard way. GLC analysis showed the absence of the p-phenylphenacyl ester of butyric acid. To the rest of the material (60%) was added 38.9 mg. (0.442 mmole) of butyric acid as an internal standard. Attempted conversion of this sample to p-phenylphenacyl esters was not successful (the pH of the solution was inadvertently not adjusted to the acidic range). The reaction mixture was made strongly acidic with sulfuric acid and distilled to dryness twice at reduced pressure (the receiving flask was cooled in an ice bath). The distillate was made basic with sodium hydroxide and evaporated. The residue was converted to p-phenylphenacyl esters in the standard manner (the pH was adjusted to about 4). GLC (6-ft. column, 3% SE-30, CT 196°, AIP 20 psi) showed among others, peaks at R.T. 7.7, 10.4, 12.1, and 14.2 min. with areas of 2.49, ca. 0.06, 0.91, and 0.93 $in.^2$ Under the same conditions the p-phenylphenacyl esters of acetic, propionic, iso-butyric, and butyric acids showed R.T. 7.6, 10.5, 12.2, and 14.5 min., respectively. These results indicated that nitric acid oxidation of 1.23 g. (ca. 1.83 mmole) of perhydro fragment had yielded 66 mg. (1.10 mmole) of acetic acid, 2.1 mg. (0.028 mmole) of propionic acid, and 37.8 mg. (0.43 mmole) of iso-butyric acid.

Perhydroflavensomycin*

Analysis of the Methyl Esters of the Nonvolatile Acids by GLC-MS. Perhydroflavensomycin (108 mg., from 114.2 mg. of flavensomycin) was oxidized with concentrated nitric acid in the usual manner. The aqueous solution was neutralized to ca. pH 1 with sodium hydroxide solution, and continuously extracted with chloroform for three days. The chloroform was dried and evaporated, yielding 60.2 mg. of yellow oil. The oil was dissolved in methanol and treated with the excess of ethereal diazomethane. After standing overnight, the solvents were evaporated, and dry benzene was distilled from the product several times. The results of combined GLC-MS analysis are summarized in Tables 14 and 15. In another oxidation, 100.5 mg. of perhydroflavensomycin yielded 62.2 mg. of yellow oil. This product was converted to methyl esters using the dimethoxypropane method.** The results of GLC analysis of this preparation are summarized in Tables 16 and 17.

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* This refers to the total reaction product from the complete reduction of flavensomycin in acetic acid using a platinum catalyst.

** See p. 39 , this thesis.

Table 14. Mass Spectral Data from Nitric Acid Oxidation of Perhydroflavensomycin. Low Temperature.*

Components in Order of Elution**	Relative Intensities of Peaks at m/e =																							
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	59	off	16	34	27	5	0	0	0	3	3	9	2	3	3	28	10	57	1	0	0	1	10	11
2	97	70	off	20	33	4	0	0	1	9	23	100	61	7	3	18	8	24	30	0	0	1	11	14
3	96	64	20	54	45	2	0	0	0	4	9	32	8	38	23	off	17	100	6	2	0	2	11	13
4	115	54	11	42	2	3	0	0	0	1	2	8	0	26	30	28	6	84	3	2	0	0	1	1
5	9	17	2	14	4	2	0	1	0	1	2	15	4	30	16	13	3	25	1	1	0	0	1	1
6	55	99	4	15	2	2	0	0	0	0	0	2	0	7	11	16	2	30	1	1	0	0	0	1
7	12	27	3	13	3	2	1	2	0	1	3	26	5	47	52	42	3	15	1	1	0	3	1	1
8	26	33	3	11	3	2	0	0	0	1	2	24	4	39	20	32	3	17	1	1	0	0	1	2
9	24	100	4	18	7	5	0	2	0	0	1	26	6	51	61	63	6	38	1	1	0	5	1	1
10	34	100	6	10	9	6	0	0	0	1	2	22	5	42	25	84	7	24	1	2	1	1	1	2
11	32	85	5	28	4	5	0	0	0	1	2	35	7	66	23	51	7	50	1	10	4	3	2	2
12	19	31	2	15	2	4	0	0	0	0	1	18	3	33	7	21	2	28	1	9	1	1	1	2
13	19	43	3	11	5	3	0	0	0	0	1	31	9	90	35	38	3	16	1	0	0	0	1	1
14	16	29	10	10	6	5	0	0	0	0	0	12	3	28	8	48	3	15	1	2	1	1	1	2
15	13	35	3	16	3	5	0	0	0	0	0	11	2	25	7	31	5	31	1	2	1	1	0	1
16	63	70	11	22	24	4	0	0	0	1	3	32	8	92	29	78	10	32	16	1	0	0	3	4
17	8	13	1	6	3	2	0	0	0	0	0	7	2	15	4	12	2	12	1	2	0	0	2	1
18	17	27	3	13	6	5	0	0	0	0	1	19	4	40	10	32	3	31	1	6	0	1	5	2
19	17	36	2	8	3	3	0	0	0	0	1	24	5	75	18	31	2	12	1	1	0	1	1	2
20	24	50	2	10	2	4	0	0	0	0	1	35	8	97	25	49	3	15	1	1	1	0	1	3

* Six-foot column, 3% SE-30, FR = 40 cc./min., operated at 40° initially and programmed to ca. 100° at about 3°/min.

** The first peak to be eluted from the the column after solvent was labeled 1, and mass spectra were taken of it and the next 19 peaks.

Table 14. Continued

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	12	1	0	0	2	26	3	100	20	9	1	2	0	0	0	0	0	0	1	1	1	4	3	1
2	13	2	1	35	15	11	2	40	8	4	1	2	0	0	0	1	1	1	3	1	1	7	4	1
3	14	5	5	16	6	24	10	63	5	20	1	3	0	3	2	3	4	13	4	6	2	7	21	20
4	2	5	4	off	39	38	8	off	8	2	1	0	0	0	0	0	1	1	0	2	10	5	4	3
5	1	1	1	11	3	5	3	100	6	2	1	1	0	0	0	1	1	18	2	2	6	5	7	2
6	1	2	1	100	27	27	3	53	4	1	0	0	0	0	0	0	0	0	0	1	3	18	3	1
7	1	1	1	71	5	3	12	100	4	1	0	0	0	0	0	0	4	10	4	2	8	3	19	2
8	1	8	8	100	29	13	7	81	3	1	0	0	0	0	0	1	1	16	1	6	8	63	12	2
9	1	2	1	63	17	12	8	95	12	7	0	0	0	0	0	2	4	27	12	5	6	24	25	5
10	2	6	5	66	26	26	13	58	7	5	0	1	0	1	1	2	3	22	4	14	4	38	16	5
11	2	17	6	78	16	21	12	72	4	5	0	0	0	0	0	4	12	47	5	24	4	58	33	23
12	2	14	2	38	10	10	10	33	2	2	0	0	0	0	0	1	2	10	2	19	2	7	4	21
13	1	9	3	57	24	24	4	85	4	1	0	0	0	1	1	9	5	100	8	8	3	57	31	4
14	1	8	3	23	8	7	9	21	2	5	0	0	0	0	0	5	4	13	2	23	3	6	10	6
15	1	7	3	25	7	6	8	17	5	9	0	1	0	1	0	2	3	17	3	19	2	9	8	9
16	4	11	6	80	32	37	8	86	7	8	0	0	0	3	2	11	14	80	11	23	4	32	40	8
17	1	3	1	11	2	4	12	18	1	1	0	0	1	1	0	3	1	10	1	3	1	2	3	4
18	1	9	2	28	4	7	36	51	2	1	0	0	2	1	0	8	3	26	2	7	2	5	4	11
19	1	10	4	86	22	29	4	56	2	2	0	2	0	2	1	9	4	69	8	5	1	32	18	2
20	2	14	5	100	21	35	4	77	3	2	0	2	0	2	2	14	6	92	11	6	2	46	28	2

Table 14. Continued

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
1	3	11	58	4	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0
2	4	11	59	5	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
3	5	13	58	5	0	1	2	3	5	8	1	38	8	8	3	4	0	0	3	1	3	2	3	6
4	0	0	2	0	0	0	1	5	2	6	7	82	6	2	0	1	0	0	0	0	0	0	0	3
5	1	1	3	1	0	0	1	1	0	5	1	14	5	1	0	1	1	0	0	0	0	0	0	2
6	0	1	2	0	0	0	1	2	1	1	1	25	24	2	0	0	0	0	0	0	0	0	0	1
7	0	1	2	0	0	0	1	1	1	8	1	30	3	1	0	1	0	0	0	1	1	10	2	2
8	0	0	2	0	0	1	9	36	3	4	2	15	35	2	0	0	0	0	0	0	0	0	0	31
9	0	0	4	0	0	1	1	1	1	13	10	37	20	2	0	0	0	0	0	1	1	12	5	8
10	1	1	2	1	0	2	5	28	5	98	6	16	30	4	1	0	0	0	1	5	1	5	2	28
11	0	1	2	1	0	6	9	25	3	off	23	21	14	10	0	0	0	0	0	1	12	13	2	5
12	1	0	2	0	0	6	2	5	2	off	19	5	7	2	0	0	0	0	0	1	2	2	1	2
13	0	1	3	3	0	4	18	57	4	28	3	14	92	5	0	0	0	0	0	4	11	11	1	9
14	0	0	3	0	0	3	3	5	1	100	7	6	5	2	0	0	0	0	0	2	2	5	2	39
15	0	1	1	1	0	2	3	7	1	100	7	7	9	6	1	2	0	0	0	1	3	5	1	32
16	1	4	9	3	3	13	15	51	8	77	5	29	100	9	2	3	1	2	1	6	6	24	6	15
17	0	0	1	1	0	1	1	2	1	100	7	3	2	2	0	0	0	0	0	2	1	2	1	3
18	0	0	2	0	0	3	3	5	2	off	21	6	3	4	0	0	0	0	1	4	2	2	2	7
19	0	2	1	3	1	11	10	49	4	10	1	17	100	6	2	1	0	1	0	5	8	51	6	7
20	0	2	1	4	1	17	15	74	6	10	1	24	off	8	2	1	1	1	1	6	11	71	8	9

Table 14. Continued.

	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
3	1	9	9	2	1	3	0	0	0	0	1	3	19	21	3	9	1	2	1	2	0	0	0	0
4	0	6	1	2	2	0	0	0	0	0	0	0	0	1	100	off	23	3	0	0	0	0	0	0
5	18	21	1	1	1	0	0	0	0	0	0	0	0	1	1	3	1	3	1	0	0	0	0	0
6	5	68	4	1	1	0	0	0	0	0	0	0	0	1	15	off	9	2	0	0	0	0	0	0
7	77	57	5	1	1	0	0	0	0	0	0	0	0	1	1	4	1	1	0	0	0	0	0	0
8	3	18	2	1	0	0	0	0	0	1	1	1	1	2	84	43	3	2	0	0	0	0	0	0
9	45	63	7	4	0	0	0	0	0	0	0	0	1	8	29	29	2	5	0	0	0	0	0	0
10	6	32	5	3	1	0	0	0	0	1	2	5	2	5	24	34	5	21	1	1	0	0	0	2
11	3	10	4	6	1	0	0	0	0	1	0	2	1	12	13	57	4	3	0	0	0	0	0	0
12	1	2	1	5	2	0	0	0	0	0	0	1	2	10	3	6	1	3	0	0	0	0	0	0
13	4	27	4	1	0	0	0	0	0	1	7	20	3	4	4	19	1	1	0	0	0	0	0	0
14	3	4	3	2	2	0	0	0	0	1	1	2	2	9	2	9	1	2	0	0	0	0	0	0
15	3	10	2	2	2	2	0	0	0	1	0	1	1	4	2	7	1	1	0	1	0	1	0	0
16	9	22	9	3	1	2	0	1	3	4	13	26	5	8	6	31	4	4	1	1	0	1	1	2
17	1	2	1	2	2	1	0	0	0	0	0	1	1	2	1	2	1	1	0	0	0	0	1	1
18	1	4	1	5	6	1	0	0	0	0	0	3	1	4	1	3	1	1	0	0	0	1	1	1
19	1	12	2	1	0	0	0	2	1	4	3	12	1	3	8	7	2	1	0	0	0	0	0	1
20	1	17	3	1	0	0	0	3	1	5	4	16	1	4	8	9	2	4	0	0	0	0	0	2

Table 14. Continued

	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
1	0	0	0	0	0	0	0	0	1															
2	0	0	0	0	0	0	0	0	1															
2	0	0	1	2	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3			
4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
5	0	0	0	1	21	33	3	1																
6	0	0	0	0	14	23	2	1	1	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	1	37	64	5	1	1	0	0	0	0	0	0	0	0	0	1	2				
8	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	18	42	3	1		
9	0	0	0	1	39	76	6	1	0	0	0	0	0	0	0	0	0	0	12	27	2	1	1	1
10	0	3	0	2	24	13	1	5	1	1	0	0	0	0	1	0	1	1	9	24	4	3	1	1
11	0	28	3	2	27	12	1	0	0	0	0	0	0	0	0	0	0	2	3	6	3	100	8	1
12	0	2	0	0	2	6	1	0	0	0	0	0	0	0	0	0	0	1	1	1	3	100	10	2
13	0	0	0	9	11	55	4	1	0	0	0	0	0	0	1	7	1	1	28	19	3	3	0	0
14	1	2	0	2	4	6	1	2	0	0	0	0	0	0	0	0	0	0	2	6	2	29	3	0
15	1	11	1	1	5	11	1	0	0	0	0	0	0	0	0	0	0	1	4	6	1	30	3	0
16	3	5	2	19	9	35	4	3	0	1	0	0	0	2	9	6	4	6	14	22	4	4	1	2
17	0	1	0	17	2	2	1	1	0	0	0	0	0	0	0	0	0	1	0	2	1	1	0	0
18	0	1	2	50	6	3	1	1	0	0	0	0	0	0	0	0	0	1	0	5	1	2	0	0
19	9	21	2	9	3	52	5	1	0	0	0	0	0	3	0	1	1	4	3	6	1	2	0	0
20	12	29	3	14	4	65	7	1	0	0	0	0	0	5	1	1	1	6	4	9	1	2	0	0

Table 14. Continued

	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171
1																								
2																								
3																								
4	0	0	0	0	0	0	0	0	0	0	0	0	0	1										
5																								
6	0	0	0	0	0	0	0	0	0	0	0	0	1	1										
7																								
8																								
9	0	0	0	0	0	0	0	0	4	8	1													
10	0	0	0	0	0	0	0	0	5	13	1	0	0	0	0	0	0	0	0	0	0	0	1	1
11	0	0	0	0	0	0	0	0	2	22	3	1	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	19
14	0	0	0	0	0	0	0	0	0	3	1	24	2	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	7	1	20	2	0	0	0	0	0	0	0	0	0	1	2
16	1	1	0	1	1	1	1	3	3	5	1	4	1	0	1	0	0	0	0	0	2	2	6	15
17	0	0	0	0	0	0	0	0	0	0	1	34	4	1										
18	0	0	0	0	0	0	1	1	1	1	2	100	10	1	0	0	0	0	0	0	0	1	0	0
19	0	0	0	1	8	2	1	1	10	15	2	3	1	0	0	0	0	0	0	0	0	3	0	0
20	0	0	0	1	8	2	1	1	14	19	2	2	1	0	0	0	0	0	0	0	0	4	1	1

Table 14. Continued

	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10	1																	
11	1	2	1															
12	2	2	1															
13	3																	
14	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
15	1	1																
16	2	0	0	0	0	0	0	0	0	0	0	0	0	2				
17																		
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
19	0	0	0	0	0	0	0	0	0	0	0	0	6	11	2	1		
20	0	0	0	0	0	0	0	0	0	0	0	0	6	13	2	1		

Table 15. Mass Spectral Data from Nitric Acid Oxidation of Perhydroflavensomycin. High Temperature.*

Components in Order of Elution**	Relative Intensities of Peaks at m/e =																							
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	20	39	29	19	4	8	0	0	0	1	1	30	7	100	21	65	6	53	3	8	1	1	3	5
5	71	95	24	19	28	8	0	0	1	2	3	45	11	off	53	off	13	68	3	3	1	1	7	11
7	59	31	6	12	18	6	0	0	0	1	2	19	6	74	18	46	9	19	1	1	0	1	4	5
13	59	62	4	9	25	2	0	0	1	1	2	21	6	off	30	off	8	18	1	1	0	1	2	3
16	90	31	7	11	43	2	0	0	0	1	1	18	5	79	15	60	13	60	1	1	0	0	3	4
17	68	28	4	7	31	2	0	0	1	1	1	13	4	71	12	68	9	51	1	1	0	0	2	3
19	65	24	6	9	28	3	0	0	0	1	2	14	3	57	12	44	13	30	1	1	0	0	3	3
29	41	36	4	10	11	5	0	0	0	1	2	16	4	82	16	50	13	67	2	3	0	0	1	3
31	35	25	3	7	10	3	0	0	0	1	1	11	3	56	10	41	10	42	1	1	0	0	1	2
33	41	41	4	13	8	7	0	0	0	1	2	20	5	99	21	66	14	95	3	3	0	0	2	2
34	64	48	8	13	17	5	0	0	1	2	2	19	5	82	19	70	20	56	2	2	1	1	3	3
35	72	48	7	15	20	4	0	0	1	1	3	26	7	99	25	78	24	88	3	2	0	1	3	4

* Six-foot column, 3% SE-30, FR = 40 cc./min., initial temperature 172°, isothermal for ca. 27 min. and then programmed at about 5°/min. to 260°.

** In total, 37 mass spectra were taken; these represent only the most intense peaks.

Table 15. Continued

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	4	19	6	75	14	22	25	57	3	8	1	1	0	3	2	25	7	42	11	55	10	17	7	25
5	7	22	15	off	38	87	30	71	5	30	2	3	1	4	3	36	17	93	20	100	13	28	off	89
7	4	8	9	100	42	27	8	61	4	3	0	1	0	3	2	12	6	50	8	24	3	13	16	6
13	3	11	10	100	27	79	17	43	2	4	1	1	1	2	1	19	9	78	13	35	4	17	off	83
16	3	13	4	61	15	26	18	34	2	3	0	1	1	4	3	28	10	45	7	52	9	21	6	5
17	2	10	3	58	15	23	24	31	2	2	0	1	0	3	2	23	9	44	6	48	8	17	4	3
19	2	8	4	49	11	22	17	46	3	4	0	1	1	2	1	15	6	48	7	30	7	32	7	39
29	2	14	6	83	16	32	29	75	4	5	1	1	1	3	2	25	9	73	10	95	15	54	9	76
31	1	6	3	59	11	26	23	45	2	4	1	1	1	2	1	20	7	53	7	54	9	36	8	38
33	2	17	7	99	22	37	48	99	5	8	1	1	0	3	2	39	11	98	14	off	28	79	9	95
34	2	13	7	85	19	43	29	65	4	8	1	1	1	3	3	31	10	82	13	91	18	81	13	47
35	2	17	8	98	22	50	57	93	6	10	1	1	1	4	3	44	17	100	16	93	22	94	20	79

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
1	2	6	12	6	2	41	7	16	11	off	12	11	24	5	2	3	1	12	3	39	6	12	6	14
5	10	16	24	14	4	61	16	52	27	37	4	off	40	5	3	4	1	14	5	51	16	42	29	15
7	3	9	12	6	2	15	14	17	53	14	3	26	28	4	2	3	1	6	3	15	5	18	23	8
13	6	5	8	6	2	22	8	40	15	36	4	off	35	3	1	2	1	6	2	20	7	29	13	7
16	1	7	8	9	3	42	9	20	6	24	4	8	17	1	1	5	1	9	4	43	14	17	6	9
17	1	6	6	6	2	39	8	17	5	20	2	6	13	1	1	4	1	9	2	38	10	13	5	9
19	2	6	6	6	2	23	7	17	6	54	6	6	12	3	1	3	1	6	3	44	8	16	7	19
29	3	7	3	12	3	59	12	25	9	off	15	12	27	4	1	5	1	25	6	63	11	21	10	29
31	2	5	2	8	2	33	9	18	6	100	9	7	14	3	1	3	1	12	4	63	11	16	7	20
33	4	8	3	14	4	92	21	32	13	off	23	15	53	5	2	7	2	24	7	92	17	29	18	40
34	3	7	4	13	4	64	16	34	12	100	14	12	26	12	1	7	2	17	7	76	16	29	14	47
35	4	10	4	18	5	77	22	40	12	off	19	14	42	7	2	5	2	26	10	off	31	36	20	42

Table 15. Continued

	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
1	6	13	2	14	7	2	0	5	2	7	5	12	5	19	4	7	1	2	1	1	0	3	2	45
5	7	32	5	5	5	11	2	8	3	15	7	29	15	33	10	34	7	5	1	2	2	12	4	20
7	6	9	3	2	2	11	1	6	2	7	6	15	6	8	8	off	98	9	1	2	2	4	3	7
13	2	25	4	3	1	4	1	8	2	7	4	14	6	7	2	11	4	1	0	1	1	4	2	11
16	2	8	1	1	1	5	1	9	2	15	4	13	4	9	2	5	1	2	1	3	1	4	3	55
17	2	7	2	1	1	3	1	7	2	11	3	10	3	7	2	4	1	2	1	2	1	4	2	49
19	3	8	2	2	1	4	1	4	3	12	4	10	4	9	3	6	2	2	1	2	1	2	3	16
29	4	12	2	2	1	6	2	10	3	14	4	16	5	35	7	8	1	3	1	3	1	14	4	35
31	2	7	2	1	1	5	1	8	3	9	3	14	11	17	3	6	1	2	1	3	1	9	3	27
33	6	16	3	2	1	8	2	16	5	25	5	21	9	62	15	10	2	2	1	5	2	12	4	63
34	6	10	8	2	1	7	3	15	5	22	7	20	11	35	9	11	2	3	1	5	2	10	5	41
35	6	17	4	2	1	11	3	18	6	25	8	32	45	26	5	9	2	3	1	5	2	14	7	65

	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
1	6	12	4	8	3	6	1	1	0	3	1	1	1	1	1	4	2	4	1	2	1	3	1	1
5	7	12	8	14	4	29	6	3	1	3	5	5	2	4	4	8	4	34	5	50	7	11	2	2
7	5	6	6	8	3	8	1	2	1	2	1	4	1	3	17	8	3	5	6	4	14	3	1	2
13	3	9	4	5	2	20	5	2	1	1	1	5	1	2	2	4	2	6	2	46	6	30	3	2
16	9	17	8	10	4	6	2	3	1	6	1	4	1	5	2	7	2	6	3	5	1	3	1	3
17	8	15	5	10	4	6	1	2	1	6	1	3	1	3	2	4	1	6	2	5	1	2	1	2
19	4	9	3	32	6	14	2	3	1	3	1	3	2	5	2	4	3	15	5	6	2	11	1	6
29	7	16	5	35	5	16	3	43	3	5	1	8	2	4	2	7	4	98	14	15	5	100	11	13
31	5	10	3	37	6	10	1	4	1	4	1	5	2	4	2	4	2	19	3	10	2	47	5	9
33	13	27	7	63	10	28	3	4	1	8	2	14	3	7	2	5	7	off	48	36	5	off	17	15
34	10	25	7	44	8	16	2	5	2	8	2	13	3	9	3	7	5	92	21	22	3	57	7	27
35	17	30	10	93	20	28	3	5	1	9	3	16	4	11	3	9	3	29	21	46	5	26	3	32

Table 15.. Continued

	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171
1	0	1	1	65	8	3	1	8	1	2	1	1	0	0	0	0	0	1	1	2	1	4	1	3
5	1	12	3	16	4	5	4	21	5	9	2	3	1	1	1	3	1	2	3	6	2	3	2	12
7	1	4	2	5	2	4	1	4	2	3	1	4	1	1	1	9	2	3	11	6	1	1	1	4
13	1	2	1	3	1	3	1	7	2	9	2	1	0	1	0	3	1	2	1	2	1	8	1	4
16	1	3	2	100	13	4	1	10	2	3	1	3	1	2	1	3	1	3	1	3	2	5	1	2
17	1	2	2	100	12	4	1	9	2	2	1	3	1	1	0	1	1	2	1	2	1	3	1	3
19	2	3	2	9	3	3	2	100	15	4	1	5	1	1	1	2	1	3	1	2	4	6	3	3
29	3	15	4	53	8	5	3	98	15	5	4	8	1	2	1	4	1	3	1	3	1	3	1	4
31	2	3	2	35	5	3	2	90	13	5	3	60	6	2	1	6	1	2	1	2	1	3	1	3
33	3	7	4	87	14	6	4	off	27	8	2	22	3	3	1	7	2	5	2	3	2	4	2	4
34	4	8	3	58	9	5	3	92	20	15	3	59	8	3	1	6	2	8	2	4	2	14	3	4
35	6	10	4	85	14	7	7	off	41	13	4	off	19	5	1	6	2	5	2	5	2	7	3	7

	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
1	4	1	1	0	0	0	1	1	1	1	1	14	2	2	0	3	0	0	0	0	0	0	0	0
5	3	19	2	1	1	2	1	2	2	4	1	4	2	13	3	10	3	1	0	2	1	1	2	2
7	1	3	13	3	1	1	1	6	2	10	2	1	2	4	1	1	1	1	1	2	1	1	1	1
13	2	2	1	1	0	1	2	2	1	4	1	1	0	7	2	7	1	1	0	1	1	1	1	1
16	1	3	1	1	1	1	1	2	1	1	1	22	4	1	1	2	1	1	1	1	1	2	1	1
17	1	2	1	1	1	1	1	2	1	2	1	21	4	2	1	2	0	0	0	0	0	0	0	0
19	1	4	1	1	1	1	1	2	1	2	2	3	3	3	2	88	12	2	1	1	1	1	1	1
29	8	87	10	2	1	11	4	2	1	7	4	13	5	6	3	86	13	3	1	4	2	2	1	3
31	2	14	2	1	1	2	1	1	1	2	3	6	3	3	3	72	10	2	1	6	2	2	1	3
33	17	off	23	4	1	2	1	2	1	4	8	25	14	16	8	100	25	4	1	13	3	2	1	2
34	7	78	11	3	1	2	1	2	2	4	5	15	5	13	5	85	15	3	1	5	2	3	1	2
35	2	19	4	3	1	4	2	3	2	4	7	26	12	17	11	off	38	6	1	5	2	3	1	2

Table 15. Continued

	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219
1	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	53	7	1	0	33
5	2	3	2	13	3	1	3	1	0	2	1	5	2	3	1	1	2	5	2	7	2	1	1	1
7	1	3	13	3	1	1	1	2	1	1	1	3	4	3	1	1	1	1	0	1	1	0	0	0
13	1	1	1	17	3	6	1	1	0	1	0	4	2	2	1	1	1	4	1	1	0	0	0	0
16	1	2	1	1	1	2	1	1	0	1	1	7	1	1	1	1	1	1	1	80	13	2	0	0
17	0	0	0	0	0	0	0	0	0	2	1	5	2	1	0	0	0	0	0	78	12	2	0	0
19	1	2	1	2	1	9	4	1	1	1	1	5	2	2	1	1	1	1	2	6	1	1	1	2
29	1	2	1	4	1	3	1	1	1	2	1	12	3	8	3	2	1	10	5	52	8	2	0	3
31	1	2	1	3	1	4	1	1	1	1	1	11	2	2	1	1	1	2	2	34	5	1	1	2
33	1	5	2	7	3	5	1	1	0	3	1	13	3	3	1	2	1	9	8	92	15	3	1	12
34	2	6	2	6	3	22	6	2	1	3	2	23	5	4	2	2	1	5	4	57	8	2	1	7
35	1	4	2	9	6	9	3	2	1	7	2	31	6	5	2	3	1	4	8	91	16	3	1	3

	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243
1	3	1																						
5	1	1	1	2	1	1	1	21	3	1	1	1	1	1	1	1	1	1	1	12	3	11	2	1
7	0	1	0	0	0	3	6	3	1	1	44	5	1	0	0	0	3	1	0	0	0	1	0	1
13	0	1	1	1	1	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	2			
19	1	4	1	1	1	1	1	1	1	3														
29	1	10	3	3	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	3	13	4	1
31	1	7	2	6	2	1	1	4	1	2	1	1	1	1	1	1	1	1	1	1	1	1	0	0
33	4	10	3	5	2	2	1	3	1	1	1	1	1	3	1	1	1	3	1	1	1	1	1	1
34	2	23	5	5	2	2	1	3	2	13	2	1	1	2	1	1	1	2	1	1	1	1	1	1
35	1	27	6	5	2	2	1	7	3	6	1	1	1	11	4	2	1	4	2	2	1	2	1	2

Table 15. Continued

	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267
1																								
5	1	1	1	38	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	0	0	0	0	0	0	0	0	0	0	5	8	1	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	17	3	0	0	0	0	0	0	0	0	0	0	9
16	0	0	0	0	0	0	0	0	0	3														
17																								
19																								
29	0	0	0	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	2	1	1	2	6	2	1	0	0	0	0	0	0	0	1	0	1
33	1	1	1	1	1	1	1	13	7	2	1	4	2	1	0	1	0	3	1	1	1	2	1	2
34	1	1	1	1	1	1	1	6	3	2	1	4	1	1	1	1	1	2	1	1	1	3	2	3
35	1	1	0	1	1	2	1	2	1	2	1	5	1	1	0	1	1	4	1	1	1	11	6	4

	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291
1																								
5	0	0	30	6	1	1																		
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1		
13	2	4	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
16																								
17																								
19																								
29	0	0	0	0	0	4	0	0	0	0	0	0	0	7	3	3	0	0	0	0	0	0	1	2
31	0	1	0	0	0	2	0	0	0	0	0	0	0	5	2	2	1	0	1	3	1	0	0	0
33	1	2	1	0	0	0	0	0	0	0	0	0	0	8	2	11	4	1	1	0	0	0	0	0
34	1	2	1	1	1	1	1	1	1	1	1	2	1	16	4	6	2	1	0	0	0	0	0	0
35	1	2	1	1	0	1	0	1	0	1	1	2	2	19	5	4	1	1	0	0	0	0	0	0

Table 15. Continued

	292	293	294	295	296	297	298	299	300	301	302	303	304	305	...	313	314	315	316	317	...	325	326	327
1																								
5																								
7																								
13	0	0	0	0	0	0	34	7	1															
16																								
17																								
19																								
29	0	0	0	3	1	1	0	0	0	2	0	0	0	0		0	0	3	0	0		0	0	0
31	0	0	0	2	1	1	0	0	0	1	0	0	1	1		0	0	1	0	0		0	0	0
33	0	4	1	3	1	1	0	0	0	4	0	0	0	0		0	3	11	2	1		4	1	1
34	1	2	1	7	2	4	2	1	1	2	1	0	0	0		1	1	4	1	0		2	1	1
35	0	4	1	7	3	11	4	1	1	0	0	0	0	0		0	0	3	0	0		4	2	2

	328	329	330	331	332	333	334	...	339	340	341	342	343	344	345	346	347	348	349	...	374	375	376
1																							
5																							
7																							
13																							
16																							
17																							
19																							
29	0	0	0	0	3	4																	
31	0	0	0	0	1	2																	
33	0	0	0	0	1	3	1		0	0	2	1	1	1	0	0	0	0	0		2	3	1
34	1	3	1	0	0	0	0		0	1	1	4	2	1	0	0	0	0	2				
35	3	10	2	0	0	0	0		1	1	5	2	2	1	1	1	2	0	0		1	4	1

Table 16. GLC Data from Nitric Acid Oxidation of Perhydroflavensomycin. Low Temperature.*

Retention Times, in Min.	Relative Area	Possible Identification Based Solely on Retention Times (as Dimethyl Esters).
6.4	10	Oxalic (6.5)**
9.4	100	Succinic (9.5, 9.6)***
10.52	17	2-Methylsuccinic (10.4, 10.6)
11.25	3	2,2-Dimethylsuccinic (11.3, 11.5)
11.8	46	
13.1	29	Glutaric (13.0, 13.2)
15.15	41	2-Methylglutaric (14.9, 15.2)
15.90	1	2,4-Dimethylglutaric, lower boiling (15.6, 16.0)
17.10	6	2,4-Dimethylglutaric, higher boiling (17.0, 17.4)
19.30	28	Adipic (19.0, 19.5)
20.40	4	
22.65	13	
23.5	31	
25.3	13	2,5-Dimethyladipic (25.2, 25.9)
29.7	3	Pimelic (30.0)**
30.9	4	
35.8	5	
37.0	9	
40.3	24	
41.7	34	

* Thirty-foot column, 3% SE-30, FR 30 cc./min., CT 136°; all retention times are from injection; standards were run immediately before and after injection for comparison of retention times.

** Retention time estimated from a semi-logarithmic plot of other straight chain standards.

*** The values given in parentheses are the retention times of those standards.

Table 17. GLC Data from Nitric Acid Oxidation of Perhydroflavensomycin. High Temperature.*

Retention Time	Relative Area	Possible Identification Based Solely on Retention Time (as Methyl Ester).
2.79	11	Pimelic (2.7, 2.7)**
3.00	86	
3.16	30	
3.30	5	Suberic (3.3, 3.3)
3.41	6	
3.73	53	
4.00	100	
4.22	3	Azelaic (4.2, 4.2)
4.64	13	
4.88	3	
5.12	2	
5.52	3	Sebacic (5.4, 5.4)
5.75	1	
6.40	1	
6.76	11	
6.91	18	
8.4	3	
9.1	2	
9.7	2	Dodecanedioic (9.6, 9.8)
10.6	2	
12.85	1	Hexadecanoic (12.7, 12.9)
15.25	5	
16.0	1	
17.9	2	
18.4	3	
20.1	3	
21.9	1	
23.2	1	
24.6	6	Octadecanoic (24.4, 24.7)
27.6	1	
29.0	1	
30.1	1	
32.8	1	
38.9	1	

* Twelve-foot column, 3% SE-30, FR 30 cc./min., CT 195°. All retention times are from injection. Standards were run immediately before and after injection for comparison of retention times.

** The values given in parentheses are the retention times of those standards.

CHAPTER III

DISCUSSION OF RESULTS

The purpose of this research was to establish as many of the structure features of the potent, toxic antibiotic flavensomycin as possible. In attacking a problem of this enormity, it was realized that there was little hope of obtaining the complete structure; degradative reactions were therefore sought with the hope of identifying some of the more simple products.

One of the first objectives was to ascertain the purity of flavensomycin as it was received. TLC analysis, by visual estimation of the intensities of separated components, indicated that the sample was about 95% pure. The impurities present were probably the simple degradation products of flavensomycin.

Several attempts were made to purify flavensomycin by standard methods. Direct crystallization of the material was very difficult and generally only led to very poor recovery of purified material. Silicic acid chromatography appeared to be somewhat successful on a small scale. However, attempted silicic acid chromatography of larger amounts (5 g.) led to nearly complete degradation of the sample. Probably the most important factor in determining the extent of degradation on silicic acid was the amount of time needed to elute flavensomycin from the column. Thus, with larger samples, a proportionately longer time was needed to elute the sample, and more degradation took place.

Small scale alumina chromatography appeared to be the best way to purify flavensomycin. When one or two per cent methanol in benzene was used for elution, most of the impurities were absorbed on the alumina and the flavensomycin was eluted quite rapidly. Probably the short contact time on the column did not allow extensive degradation to take place. Flavensomycin could easily be crystallized after alumina chromatography and did not show any detectable impurities by TLC analysis.

Flavensomycin was not very stable in many common organic solvents; it was extensively degraded in all solvents tested within one week's time at room temperature. It was degraded particularly rapidly in acetic acid and pyridine, and moderately rapidly in chloroform, methylene chloride, ethyl acetate, and tetrahydrofuran. The degradation of flavensomycin, in all of these solvents, produced at least two components; one had R_F 0.00 (the R_F value of flavensomycinoic acid was 0.00 in this solvent system) and the other had a high R_F value. Flavensomycin was most stable in benzene solution.

The products derived from the degradation of flavensomycin in boiling chloroform were investigated. In one study, complete degradation of flavensomycin had taken place in one day as evidenced by TLC analysis. In another study only partial degradation took place after two days. This may have been due to differences in the purity of the chloroform used. The product from the later study was separated into three main components by chromatography on unisil. The first fraction (high R_F component) was probably the fragment of flavensomycin left after flavensomycinoic acid had been released. However, the ultraviolet absorption (λ_{max} 225 and 312 mμ, $\varepsilon \cong$ 11,200 and 970) was very different than that of the difference in

ultraviolet absorption between flavensomycin and flavensomycinoic acid (λ_{max} 250 and 282 mμ, see Figure 6). This indicated that some rearrangement of the chromophoric unit had taken place during the chloroform degradation.

The next main fraction appeared to be flavensomycin as judged by TLC analysis and its ultraviolet absorption spectrum.

The last fraction was probably flavensomycinoic acid because of the similarity of both its ultraviolet spectrum and TLC behavior when compared with an authentic sample.

Several physical properties of flavensomycin have been reported in the literature. Some of these determinations have been repeated, and in particular the infrared and ultraviolet spectra agree well with values reported in the literature (1,4,5,6). The melting point of the sample of flavensomycin after crystallization was 141-143.5°. Two values of the melting point have been reported in the literature, namely 130-131° and 152 ± 2° (1,4). The discrepancy in the melting point behavior of flavensomycin was probably due to its extreme ease of decomposition. In general, the observed melting point of a heat sensitive compound will depend strongly on the rate of heating and the actual method used for the determination. Since these factors were not known for the literature melting points, it was felt that the lack of quantitative agreement did not cast any serious doubt on the purity of the flavensomycin used in these experiments.

The n.m.r. spectrum of flavensomycin showed, among other absorptions, two singlets at -3.50 and 0.83 τ, each of which corresponded to one proton. The absorption at -3.50 τ probably corresponded to a strongly acidic, enolic proton since they commonly absorb in this region (49).

Since this proton was not rapidly exchanging with other hydroxyl protons it was probably intramolecularly hydrogen bonded. This absorption could be attributed to the enolic hydroxyl group of flavensomycinoic acid, if of course, it were free in flavensomycin. Other evidence* indicated that the enolic hydroxyl group is free. The absorption at 0.83 τ probably corresponded to the amide proton of the flavensomycinoic acid portion (the amide proton of flavensomycinoic acid absorbed at 0.10 τ in dimethyl sulfoxide-d_6). Both of these signals disappeared after the deuteriochloroform solution was equilibrated with deuterium oxide. The remaining absorptions are not easily assignable to specific groups. Part of the absorption in the region 2.33-3.83 τ was probably due to the olefinic protons of the flavensomycinoic acid fragment (they absorb at 2.56 and 3.33 τ in dimethyl sulfoxide-d_6). The proton count in the olefinic region (2.33-5.58 τ) indicated that there were approximately nine olefinic protons in flavensomycin (actual value obtained, 9.05). Methoxyl group commonly absorb in the region 6.0-6.7 τ (49). There were several sharp absorptions in this region of the spectrum. The proton count indicated that there was a maximum of three methoxyl groups. Since other protons might absorb in this region, this value compares favorably with the value determined by analysis (2.62 groups, indicative of three methoxyl groups).

The absorption in the region 7.67-8.50 τ contained about 14 hydrogens by integration. This absorption was characterized by a strong peak centered at 8.04 τ. In light of information later derived, it is suggested that this absorption results from methyl groups attached to unsaturated

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* See p. 93, this thesis.

carbon. Such groups are reported to absorb in the region 8.1-8.4 τ (49). From the integration, it is suggested that there are four such groups.

The absorption in the region 8.50-9.67 τ contained about 25 hydrogens by integration. Absorption in this region results only from methylene and methyl groups attached to saturated carbon (49).

The other absorptions present could not be readily assigned.

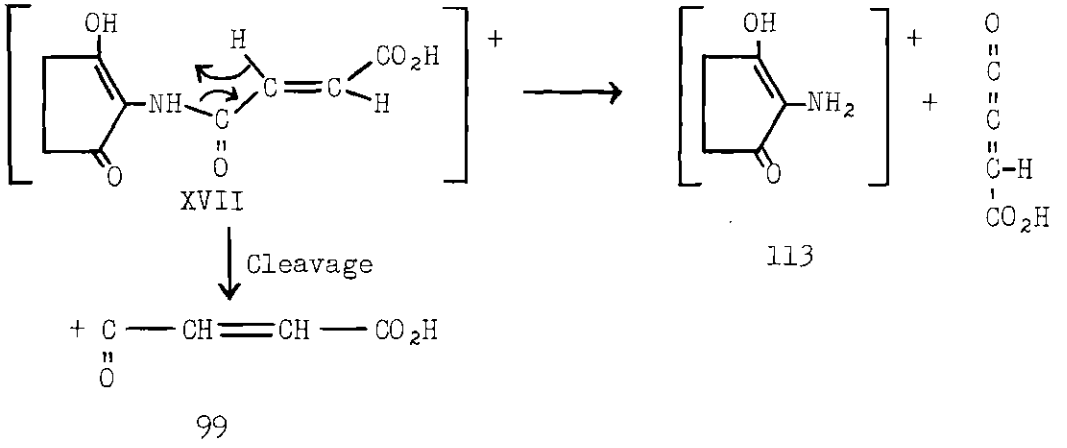
The formula $C_{38}H_{58}N_2O_{10}$ has been proposed for flavensomycin (4). This formula is probably in error, since the percentage of nitrogen is approximately twice that reported by other workers (5,6,7). The analytical results that have been obtained, both for stock and purified flavensomycin, were in substantial agreement with those reported in the literature. These results are, of course, consistent with several empirical formulas, depending on the molecular weight.

Several attempts were made to determine accurately the molecular weight of flavensomycin. Vapor pressure osmometry is one of the quickest and easiest methods for the accurate measurement of molecular weights. Using this method (with ethanol as solvent), values of 849 and 846 were obtained for two different samples of purified flavensomycin. Values obtained from known standards indicated that this method was accurate to within at least $\pm$ 5%. Thus the molecular weight of flavensomycin, as determined by vapor pressure osmometry, was 848 $\pm$ 42.

The mass spectrum of flavensomycin (Figure 3) showed, among others, a very weak peak at m/e ca. 870 as the highest observable peak. Because of the very low background in this region of m/e values, an accurate mass count was impossible. However, the instrument was equipped with an automatic mass marker. This mass marker was accurate in the region of the

spectrum that could be counted with assurance. The highest peak appeared to have an m/e value of 871. Thus, the molecular weight of flavensomycin would be 872. The formula $C_{47}H_{69}NO_{14}$ (872.07) gives good agreement between the calculated and found values for carbon, hydrogen, nitrogen, and oxygen.

In addition, the mass spectrum of flavensomycin showed a strong peak at m/e 211, which indicated that flavensomycinoic acid (XVII) (molecular weight, 211) was easily eliminated. Several other strong peaks may be related to flavensomycinoic acid as shown below. The peak at m/e 166



may result from loss of 45 mass units ($-CO_2H$) from the peak at m/e 211. The strong peak at m/e 113 corresponds formally to the loss of the cyclopentanedione ring, plus one hydrogen from the molecular ion of XVII. It is also possible that this ion was not derived from the flavensomycinoic acid fragment. Many of the other peaks probably are derived from the other portion of flavensomycin (those above m/e 211 must come from this part). Interpretation of these peaks did not appear to be possible. It was thought that there might be a peak at (M-211) corresponding to the loss of flavensomycinoic acid. However, the peak at highest mass (before

the molecular ion) was at m/e 568 which corresponded to the loss of 303 mass units.

The lactone titration of flavensomycin gave an equivalent weight of 216. If there are four groups in flavensomycin which are able to consume base, then the calculated equivalent weight is 218 (based on molecular weight 872). This excellent agreement supported the proposed formula.

In addition, Kuhn-Roth $C\text{-}CH_3$ determinations gave a value of 11.2% (average of two). This is to be compared to a literature value of 10.3%. The value of 11.2%, indicated 6.51 $C\text{-}CH_3$ groups (based on molecular weight of 872). In general, the yield of volatile acids by the Kuhn-Roth procedure is about 65%. This would indicate that flavensomycin contains about 10 $C\text{-}CH_3$ groups, depending on the assumed yield. The literature value of 10.3% was taken as indicating three $C\text{-}CH_3$ groups. Based on their assumed molecular weight (703), 10.3% calculates to 4.83 $C\text{-}CH_3$ groups. Assuming a yield of 65%, this would indicate about seven to eight $C\text{-}CH_3$ groups. Apparently, these quantities were not correctly calculated.

An $O\text{-}CH_3$ analysis gave a value of 9.34% as compared to the literature value of 9.18%. The value of 9.34% calculates to 2.62 groups and indicates that flavensomycin probably contains three $O\text{-}CH_3$ groups. The literature value of 9.18% calculates to 2.08 $O\text{-}CH_3$ groups (assumed molecular weight, 703) and was taken to indicate two $O\text{-}CH_3$ groups.

An active hydrogen analysis (done in anisole as solvent) gave a value of 0.855%, which calculates to 7.45 groups. Since it is very difficult to obtain quantitative results on polyhydroxy compounds, this result would indicate at least eight to ten active hydrogens.

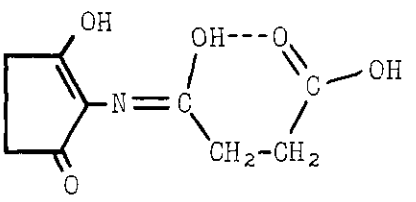
An $N\text{-}CH_3$ analysis (0.86%, 0.26 group) showed that flavensomycin,

as expected, did not contain any such group.

Flavensomycin possesses a strong ultraviolet chromophore; about one-third of the absorption can be accounted for by the flavensomycinoic acid fragment. The absorption was, however, not typical of a polyene system (as in the polyene macrolide antibiotics), which have a series of sharp maxima (50). In contrast, the ultraviolet spectrum of flavensomycin shows just one broad maximum with a shoulder on the long wavelength side. When a plot was made of the difference of extinction coefficients between flavensomycin and flavensomycinoic acid versus wavelength, the result should approximate the spectrum of flavensolids (i.e., flavensomycin minus the flavensomycinoic acid fragment). This plot reveals two maxima at 250 and ca. 282 mμ (ε 39,000 and 16,800), as is shown in Figure 6.

The ultraviolet absorption of flavensomycin changes in 1 N ethanolic hydrochloric acid. Immediately there was a small hypsochromic shift and a small decrease in extinction. The main absorption band continued to shift to shorter wavelength (up to 72 hr.) and then started to shift to longer wavelength. The extinction coefficient decreased up to the longest time tested. At the same time, the shoulder became more pronounced and then changed to a definite maximum whose position remained constant. The extinction coefficient increased slowly up to about 72 hr., abruptly increased, and then decreased. These changes in the ultraviolet spectrum of flavensomycin can probably be ascribed to two main causes: protonation of the flavensomycinoic acid portion and its subsequent hydrolysis, and acid catalyzed reactions of the other fragment (isomerizations and/or dehydrations).

The $\underline{p}K_a$ value of flavensomycin was 5.8 when determined in 3:1 ethanol-water (4). This is to be compared to the $\underline{p}K_a$ of flavensomycinic acid (XIII), 5.64, when determined in the same solvent system (8,9). this close correspondence of $\underline{p}K_a$ values suggested that the acidic group in flavensomycin was the enolic hydroxyl group of the flavensomycinoic acid portion. The $\underline{p}K_a$ values of flavensomycinoic acid were 3.5 and 4.7 (water) (8,9). The value of 3.5 probably corresponded to dissociation of the carboxyl group [fumaric acid, $\underline{p}K_a$ 3.02, 4.38 (water) (51)] and the value of 4.7 to the enolic hydroxyl group. It is generally found that acids are stronger in water than in alcohol-water mixtures (52); this accounts for the decreased $\underline{p}K_a$ values in water. The reported $\underline{p}K_a$ values of dihydroflavensomycinoic acid (XVI) are 2.8 and 3.6 (water) (8,9). These are, however, subject to doubt because the pure acid was not isolated. The $\underline{p}K_a$ values of dihydroflavensomycinoic acid, as determined in this work, were 3.07 and 4.70 (water). The value of 4.70 was in perfect agreement with the $\underline{p}K_a$ assigned to the enolic hydroxyl group in flavensomycinoic acid. Therefore, the value of 3.07 would be the $\underline{p}K_a$ of the carboxyl group of XVI. However, the reported $\underline{p}K_a$ of succinamic acid is 4.54 (water) (53). A possible explanation of this large increase in acidity may be the formation of an internally hydrogen bonded species, as shown below. The driving force for this reaction, as compared



to succinamic acid, would be the increased conjugation that would result.

In order to test the possibility of some easily reducible functional group in flavensomycin, sodium borohydride reduction was attempted. Of the material used, 76% (by weight) was recovered in the benzene-soluble fraction. The remaining 24% could not be recovered. TLC indicated that the benzene-soluble material was a mixture of at least five different components. The infrared spectrum of this mixture showed the presence of hydroxyl (2.88 μ), carbonyl (5.85 μ), and carbon-carbon double bond (6.17 μ) absorption. Significantly, absorption at about 3.08 μ (N-H of amide) was absent. The ultraviolet spectrum of this mixture was quite similar to that of flavensomycin except that absorption at 273 mμ was a definite maximum, and not a shoulder as in flavensomycin. The n.m.r. spectrum of this substance was indicative of a mixture; all the absorptions were broad and poorly resolved. The spectrum did indicate that olefinic, methoxyl, and saturated aliphatic protons were present.

This evidence indicated that flavensomycin had been degraded in ethanolic sodium borohydride with the release of the flavensomycinoic acid fragment. The remaining portion of the molecule was then degraded and/or isomerized under these conditions. Because of the complexity of the benzene-soluble fraction, it was not investigated further.

Methanolysis of flavensomycin was carried out according to Canonica et al. (7,8). The reaction was followed by TLC, which indicated that little, if any, flavensomycin remained after four days. The objective of this experiment was to investigate the other products that are formed besides flavensomycinic acid. The hexane-soluble material (flavensomycinic acid is not appreciably soluble in hexane) was chromatographed on

alumina. Two main fractions were obtained; both were complex mixtures of at least four components as judged by TLC. The infrared spectra of these two fractions were quite similar and showed hydroxyl (2.83 μ), carbonyl [5.75 (sh.), 5.81, and 5.92 μ], and carbon-carbon double bond (6.18 μ) absorption. Due to the complex nature of these fractions they were not investigated further. Apparently the acidic conditions necessary to produce flavensomycinic acid cause extensive degradation and/or isomerization in the other part of the molecule.

The reduction of complex unsaturated molecules is a very common technique used in structure determinations. Often, the reduction product is considerably more stable, relative to the unsaturated compound, and consequently more amenable to investigation. Since flavensomycin was very unstable, it was hoped that a study of its catalytic reduction would be fruitful.

When flavensomycin was reduced in acetic acid solution, using 5% platinum on carbon as catalyst, about seven moles/mole of hydrogen were consumed (the average of three determinations was 6.97). The reaction proceeded at almost a constant rate up to about four moles/mole; this indicated there were probably four similar reduction sites (such as four similarly substituted double bonds). The reduction proceeded much slower beyond this point. Possibly other types of reducible groups, such as epoxides, carbonyls, or more hindered carbon-carbon double bonds, were being reduced. Reduction of flavensomycin using ethanol as the solvent did not take the same course as when acetic acid was used. Only about four moles/mole of hydrogen were consumed. In addition, the yield of dihydroflavensomycinoic acid was much lower.

The product from hydrogenation in acetic acid was chromatographed on silicic acid. This yielded two main fractions. The first one was a colorless glass and comprised about 78% of the total weight. This material was called the perhydro fragment. After chromatography on alumina, this material was homogeneous as analyzed by TLC. An infrared spectrum of the perhydro fragment indicated that it contained hydroxyl (2.78 μ) and carbonyl (5.74 μ) absorptions. There was no λ_{max} at 5.74 μ in the infrared spectrum of flavensomycin, but a shoulder at about 5.75 μ was present. The positions of this absorption was typical of esters or lactones (δ or larger). Strong absorption at this wavelength would have been expected if the perhydro fragment contained a macrocyclic lactone ring (i.e., if flavensomycin was a macrolide antibiotic); however, the possibility of a simple ester function cannot be excluded. The n.m.r. spectrum of the perhydro fragment was indicative of a mixture since the absorptions present were broad and poorly resolved. The position of these absorptions was typical of a molecule containing mostly saturated aliphatic protons (8.6 and 9.1 τ). The weak absorption at 6.7 τ could be attributed to methoxyl groups. The GLC behavior of the trimethylsilyl derivative of the perhydro fragment was very complex, exhibiting at least eight peaks. However, this could have been caused by either incomplete reaction and/or decomposition at the required column temperature. It is also possible that the catalytic hydrogenation had produced a mixture of stereoisomers.

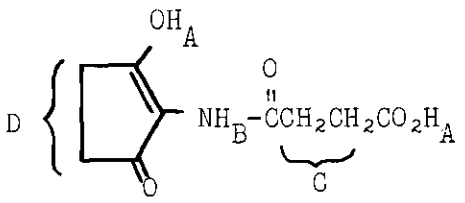
Nitric acid oxidation of the perhydro fragment produced about 3.3 moles/mole of carbon dioxide, assuming that the molecular weight was about 671. Probably little quantitative significance can be attached

to this number. However, it was considerably smaller than the difference in the moles/mole ratio of flavensomycin (ca. 13.4) and flavensomycinoic acid (ca. 7.5) (difference about six moles/mole). In addition, the volatile acids from the nitric acid oxidation were determined by GLC analysis of the p-phenylphenacyl esters. An internal standard was used in an attempt to estimate the actual yield of acids. However, since the first attempt to convert the mixture to p-phenylphenacyl ester was not successful (the pH was not adjusted correctly), the quantitative nature of the results are doubtful. The GLC analysis indicated that about 0.6, 0.02, and 0.24 mole/mole of acetic, propionic, and isobutyric acids, respectively, were produced.

The other main component (20%) was a white crystalline compound. This material was shown to be dihydroflavensomycinoic acid (XVI). Although this compound has been described in the literature, it was not isolated. The only physical properties of this compound that are described in the literature are the pK_a values. Potentiometric titration of a sample gave values of 3.07 and 4.66 (water) as compared to the literature values of 2.8 and 3.6 (water) (8,9). The agreement, particularly between the higher pK_a values, was not very good. However, the authors did not state the method used in their determination.

The analytical data, n.m.r. and infrared spectra are in excellent agreement with the proposed structure. The n.m.r. data (pyridine solution are assigned by the structural formula, as shown below.

The ultraviolet spectrum of XVI was quite informative in that it showed a strong pH dependence (see Figure 4) and had an isosbestic point at 248 mμ. The isosbestic point indicated that one of the dissociating

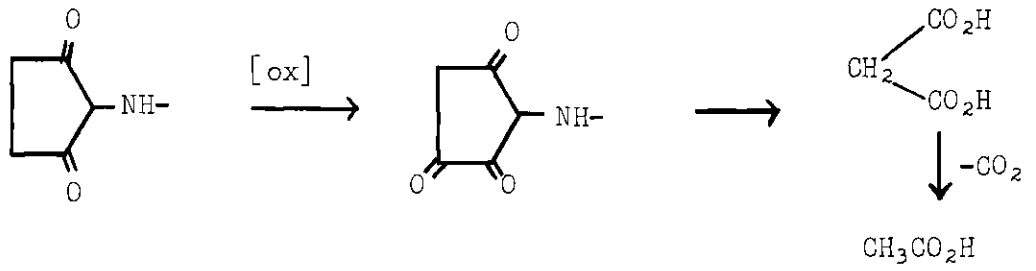


XVI

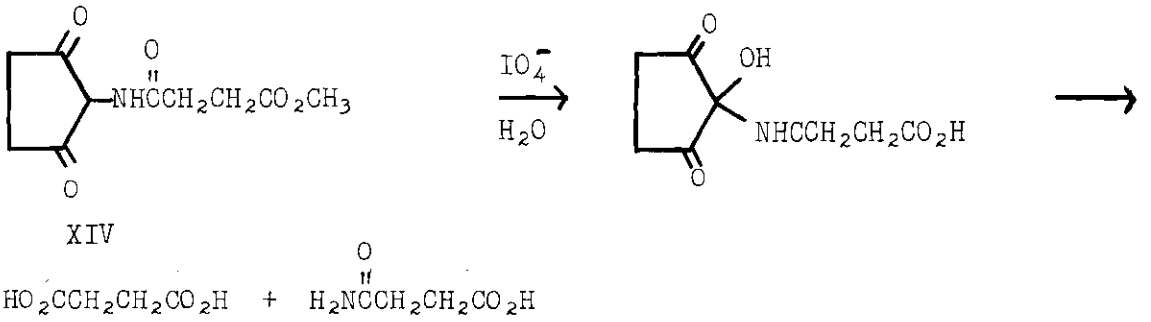
Proton	τ
A	-3.10
B	-0.27
C	7.00
D	7.66

groups was associated with the chromophore. An attempt was made to obtain a 2,4-dinitrophenylhydrazone derivative of XVI, but as reported, no pure product could be isolated.

A Kuhn-Roth determination on dihydroflavensomycinoic acid gave a value of 2.17%, which calculates to 0.31 group. Since the proposed structure, as shown by synthesis, does not have a C-CH_3 function some unusual oxidation must have taken place. Perhaps (at least in part) the cyclopentanedione ring was being oxidized as shown below. If malonic acid was formed, it would be decarboxylated under the acidic conditions and acetic acid would be formed. This might also explain the formation of acetic acid from flavensomycinic acid, as reported by Canonica et al., and as shown by GLC analysis of the p-phenylphenacyl esters from nitric acid



oxidation. Canonica et al. also reported the formation of propionic acid by the vigorous basic hydrolysis of dihydroflavensomycinic acid, followed by oxidation with hydrogen peroxide. This was offered as evidence for the originally proposed structure (IV). Since the structure has since been revised to XIV, it is very difficult to rationalize the formation of propionic acid. The identification was, however, based solely on paper chromatographic analysis and possibly was in error. They also reported the formation of oxalic and succinamic acids by periodate oxidation of dihydroflavensomycinic acid. The formation of oxalic acid is difficult to rationalize on the basis of either of the proposed structures. Presumably, succinamic (the methyl ester probably is hydrolyzed) and succinic acids should have been produced, based on structure XIV. Again, the



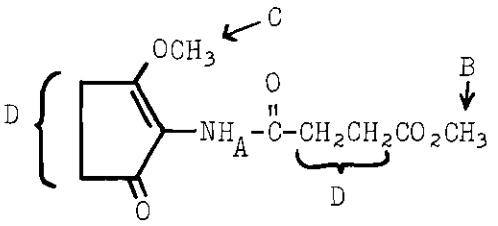
XIV

$HO_2CCH_2CH_2CO_2H$ + $H_2NCCH_2CH_2CO_2H$

identifications were based on paper chromatographic analysis and could have been in error.

The reaction between XVI and an excess of diazomethane produced methyl dihydroflavensomycinate (XV). The analytical data, n.m.r., infrared, and ultraviolet spectra of this compound are in complete agreement with those recorded in the literature and strongly support the proposed structure. The n.m.r. data are assigned by the structural formula as

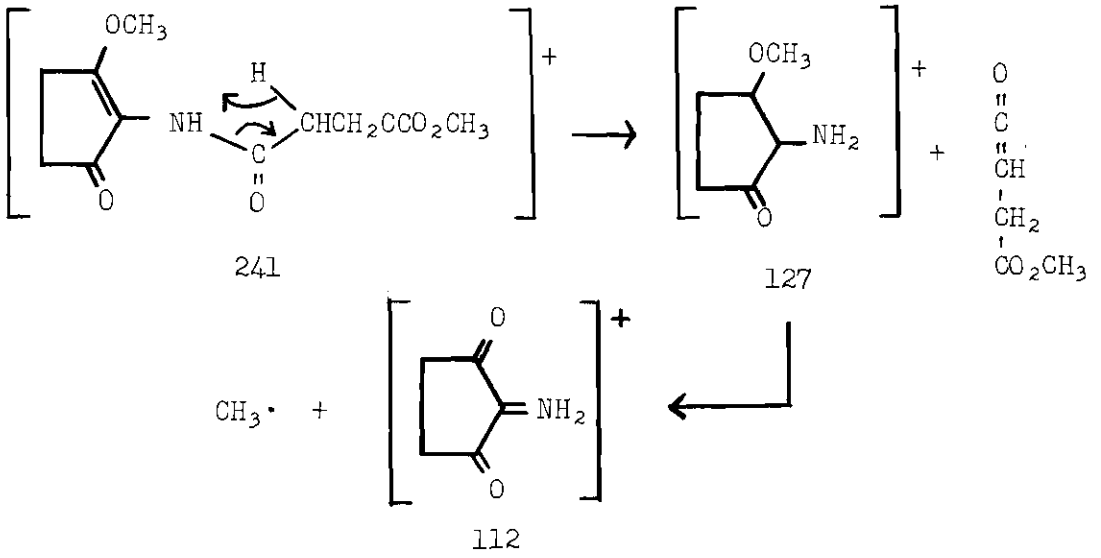
shown below. In addition, the mass spectrum of XV (Figure 5) can easily



XV

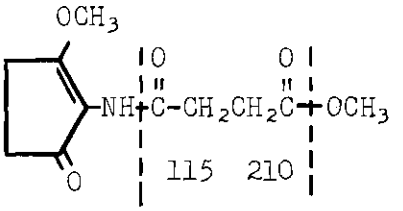
Proton	τ
A	2.07
B	5.92
C	6.30
D	≈7.33

be rationalized in terms of this structure. It showed a molecular ion peak at m/e 241, and prominent peaks at m/e values of 210, 127 (base peak), 115, 112, and 55. The base peak at m/e 127 is thought to arise by the following mechanism which then by simple cleavage can yield an ion with m/e 112. The other peaks, at m/e 210 and 115 could arise as

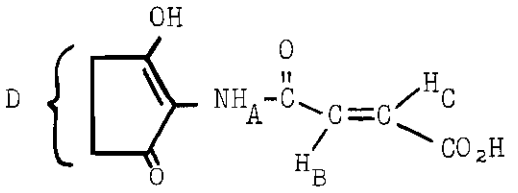


241 127 112

shown below by simple cleavage reactions. The ion with m/e 55 probably is $(O{=}C{=}\underset{\displaystyle H}{\underset{|}{C}}{-}CH_2]^+$, formed by loss of methanol and cleavage.

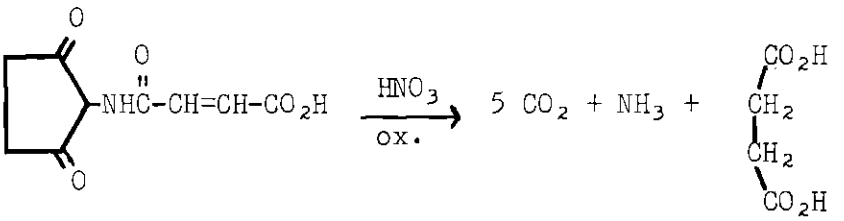


Part of the material from the degradation of flavensomycin on silicic acid (that which was most strongly adsorbed on the column) was shown to be flavensomycinoic acid (XVII). The melting point of this sample agreed with the literature value. The infrared and ultraviolet absorption spectra of this compound are not reported in the literature, but the values obtained are consistent with the proposed structure. The n.m.r. data, as summarized below, were also consistent with the proposed structure.



Proton	τ Value	J (cps)
A	0.10	J_{BC} = 15
B	2.56	
C	3.33	
D	7.50	

Nitric acid oxidation of flavensomycinoic acid produced acetic acid (as discussed previously) as well as about 7.5 moles/mole of carbon dioxide. It was expected that about 5 moles/mole of carbon dioxide would be produced, as shown below. Therefore, either further oxidation of



succinic acid, or the other types of oxidative pathways must have taken place.

It was hoped that nitric acid oxidation of flavensomycin and perhydroflavensomycin would produce relatively simple mixtures of compounds, most of which were expected to be dicarboxylic acids. It was therefore necessary to prepare a series of dicarboxylic acid and convert them to their methyl esters. The methyl ester standards were prepared from the corresponding acids by the dimethoxypropane (54) method. This procedure worked quite well for all of the acids with the exception of diethylmalonic. Apparently this acid is too sterically hindered for this type of esterification reaction. It was easily converted to its methyl ester by the use of diazomethane. The yields of esters from β-methyltricarballylic and dimethylmalonic acids also appeared to be quite low. Again, this was probably due to steric hindrance. Several of the acids (purchased) were shown to be mixtures by GLC analysis and their melting point behavior. Thus, 2,3-dimethylsuccinic and 2,4-dimethylglutaric both showed two well resolved peaks by GLC analysis of their methyl esters. The melting point ranges of these acids were broad and corresponded to mixtures of dl and meso isomers. meso-2,3-Dimethylsuccinic acid was synthesized by catalytic hydrogenation of 2,3-dimethyl maleic anhydride. GLC analysis of this compound showed only one peak; this peak corresponded to the component of shorter retention time (lower boiling) as compared to the mixture of dl and meso methyl esters. The melting point behavior of the 2,3-dimethylglutaric acid received indicated that it was a mixture of the two dl pairs. GLC analysis of the methyl ester preparation of this acid showed only one (broadened) peak. GLC analysis of the

dimethyl 2,5-dimethyladipate received also showed one (broadened) peak, indicating that it was probably a mixture of isomers.

The preparation of p-phenylphenacyl ester standards was quite straightforward and gave good yields for all of the acids used. The products were easily purified by recrystallization if freshly recrystallized p-phenylphenacyl bromide was used.

It was thought that hot, concentrated nitric acid would attack all easily oxidizable functional groups. Therefore any acids produced by the oxidation of flavensomycin or perhydroflavensomycin would not be expected to contain carbonyl (aldehyde or ketone), carbon-carbon double bond, or hydroxyl groups. Methoxyl groups (ether linkages) were, however, expected to be stable to these conditions; methoxy acids were therefore expected in the mixtures of products.

Since methoxy acids were expected to be products, it was envisioned that some difficulties might arise in interpreting the mass spectral results. A $-\overset{|}{C}HOCH_3$ group has the same mass as a $-\overset{O}{\overset{\|}{C}}-O-$ group (44 mass units). Thus, for example, methyl methoxypenanoate has the same molecular weight (146) as does dimethylsuccinate. In general, the methyl ester of a C_n methoxy carboxylate has the same molecular weight as the methyl ester of a C_{n-1} carboxylate containing one less methoxyl group and one more carboxyl group. In addition, a C_n-tricarboxylate has the same molecular weight as a C_{n+2}-dimethoxyl monocarboxylate (all as methyl esters).

Metastable peaks were often used to confirm the relationship between peaks. For example, if two peaks differed by 28 mass units the observance of a metastable peak corresponding to the transition

$RCO^+ \rightarrow R^+ + CO$ would show the relationship of the two ions (i.e., that they were not just accidentally 28 mass units apart). For the transition $m_1^+ \rightarrow m_2^+ + (m_1 - m_2)$, the calculated value for the metastable peak (m^*) was found using the equation, $m^* = \frac{m_2^2}{m_1}$ (55). The absence of metastable peaks does not, however, preclude the possibility that two ions are related (55).

It was also observed that the mass spectra of the standards were not completely reproducible. This may have been in part due to several reasons: a pressure dependence (the actual amount of sample in the ionization chamber) may change the relative importance of intermolecular reactions and concentration gradients which occurred during the determination, caused by the length of time necessary to record the mass spectrum, may be important in certain cases. Thus allowances must be made for small changes in relative intensities when comparing the mass spectrum of a standard to that of an unknown component.

The nitric acid oxidations of flavensomycin and perhydroflavensomycin yielded about 22% and 59% (weight basis) of nonvolatile products, respectively. The expectation that these products would be relatively simple mixtures was not realized; both mixtures contained many (ca. 40) components when analyzed as methyl esters. The analyses of these mixtures by gas chromatography were not completely satisfactory. The complexity of the mixtures, coupled with the fact that there was no way to guarantee that all of the components were simple methyl esters, made identifications rather tenuous. However, by the combined techniques of GLC and MS, it was hoped that much more information could be gained about the nature of these mixtures. To this end, it was first necessary to

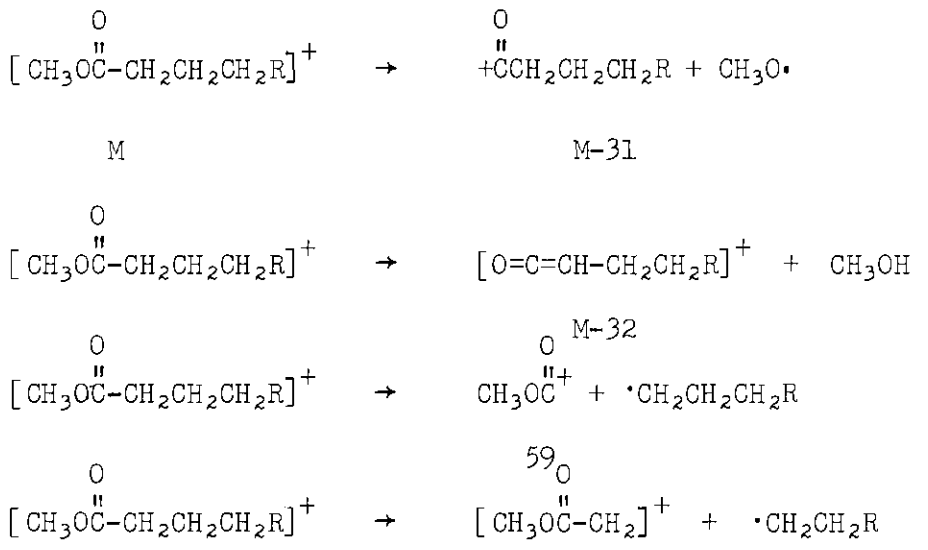
determine the mass spectra of a number of known methyl esters. Thus, spectra would be available not only for direct comparison, but also hopefully they would aid in the understanding of the mechanisms by which methyl esters fragment under electron impact.

Esters, and particularly methyl esters, have been the subject of many mass spectral investigations. Most of the important processes by which these compounds fragment are reasonably well understood and are summarized completely in several recent books (56,57). As with most compounds, the primary process is the formation of the molecular ion (M^+).

$$M + e^- \rightarrow M^+ + 2e^-$$

Most of the monocarboxylic esters show molecular ion peaks, whereas the dicarboxylic esters show no, or only very weak molecular ion peaks. As with many compounds that contain oxygen and/or double bonds, most of the intense peaks contain these functions. Some of the favored simple fragmentation mechanisms are shown below. These types of mechanisms will in general account for the most intense peaks in the mass spectra of methyl esters. There are several additional considerations which must be taken into account: branching of the chain and substitution by groups other than hydrocarbon.

In general, branching of the chain will have a pronounced effect on the mass spectrum. A methyl group in the α-position will shift the strong peak at m/e = 74 to m/e = 88. It is also observed that there is much more cleavage next to branching points as compared to the straight chain compound. Thus, strong peaks due to simple cleavage reactions often indicate a branching of the chain.



M

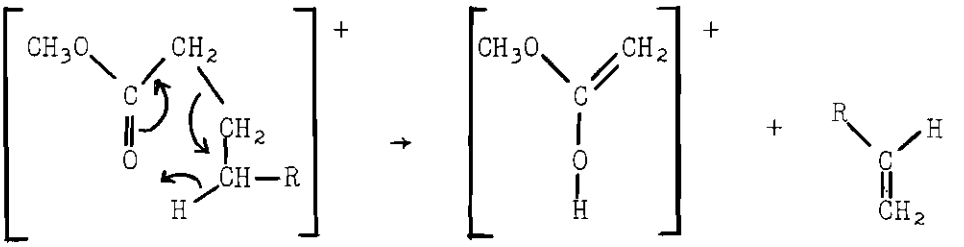
M-31

M-32

73

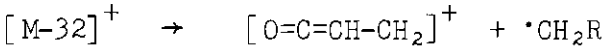
59

Series of peaks at 73,87,101,... depending on site of cleavage and substitution



74

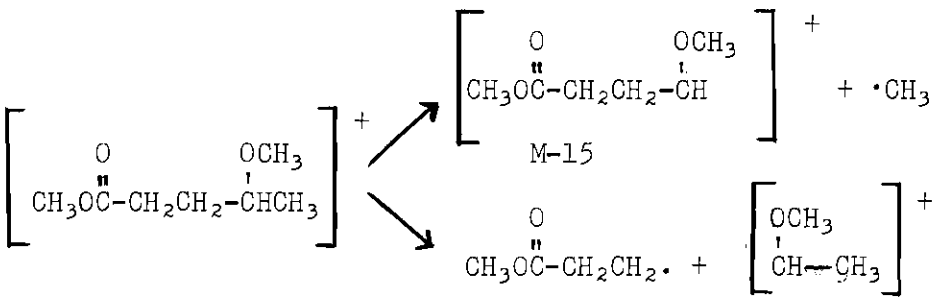
88 if α-Methyl
102 if α,α-Dimethyl;
other values depending on substitution



55

Series of peaks at 55,69,83,... depending on site of cleavage and substitution

In addition, other types of substituent groups may have a profound effect on the spectrum. Often these groups, such as methoxyl, can greatly enhance the ease of simple cleavage reactions, as shown below. Again,



59

very intense peaks, due to this type of simple cleavage, are usually observed.

The mixtures of nonvolatile products obtained from nitric acid oxidation of flavensomycin and perhydroflavensomycin were converted to methyl esters using diazomethane. These mixtutes of methyl esters were analyzed by GLC-MS. The discussion of the data obtained by this technique is organized in a similar manner as to the Tables 10, 11, 14, and 15. It should be pointed out that the sets of tables, unfortunately, do not overlap (i.e., for both the flavensomycin and perhydroflavensomycin data, none of the spectra recorded during the low temperature runs is in the high temperature runs).

The data in Table 10 are from the low temperature flavensomycin runs. To facilitate discussion, individual spectra will be referred to by first the component number and then the Table number (i.e., the mass spectrum representing component No. 1, Table 10, will be spectrum 1-10). In addition, whenever a compound is referred to, it is assumed to be the

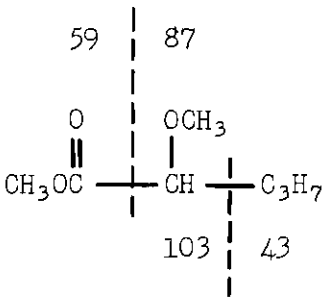
methyl ester unless otherwise stated (i.e., succinate refers to dimethyl succinate, and 2-methylsuccinate refers to dimethyl 2-methylsuccinate).

Spectrum 1-10 appeared to be somewhat consistent with oxalate. Although a spectrum of oxalate was not obtained, it has been reported in the literature (56). Thus the base peak at m/e 59, strong peaks at m/e 29 and 45, and the molecular ion peak at m/e 118 were consistent. Other peaks which were not consistent were at m/e 78, 91, 92, and 106. These peaks could be due to benzene (solvent), toluene, and xylene (impurities in solvent).

Spectrum 2-10 had as its base peak m/e 59, which is a characteristic ester peak ($CH_3O\overset{O}{\overset{\|}{C}}+$). In addition, peaks at m/e 45 and 29 were very strong. There was a very weak peak at m/e 132, which is the molecular weight of malonate. However, it is very doubtful if malonic acid could have survived the nitric acid oxidation. The spectrum did not fit the published one (56) for malonate and did not appear to be any easily recognizable methyl ester. Spectrum 3-10 was similar to No. 2-10.

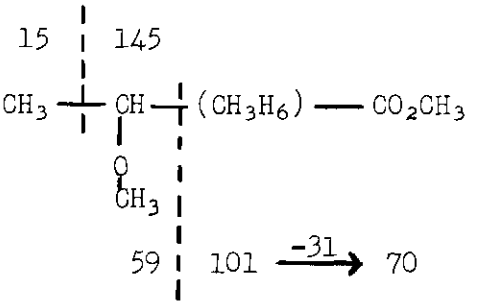
Spectrum 4-10 was quite similar to that of succinate except for the peaks at m/e 43 and 103. Possibly this was a mixture of succinate and some other ester, although the GLC peak was quite symmetrical. It is also likely that this is not succinate, but a mono-methoxy mono-carboxylate (i.e., mono-methoxypentanoate, MW = 146, M-31 at m/e 115; these types of compounds will be denoted as, for example, C_5-OCH_3 MC, a more complete list of abbreviations is given on p. x). The only observable metastable peak was at m/e 65.9, which corresponded to the transition $115^+ \rightarrow 87^+ + 28$ (calculated, $m^* = 65.8$). This was simply loss of carbon monoxide from the M-31 peak. It is interesting to note that the two

peaks that do not fit for succinate, m/e 43 and 103, add up to 146. A possible structure might be the one shown below. This structure seems to be in good agreement with the observed mass spectrum (or possibly this



compound mixed with succinate).

The strong peak in spectrum 5-10, at m/e 129, corresponded to a C_5-DC or a C_6-OCH_3 MC. The spectrum was not consistent with 2-methylsuccinate, dimethylmalonate, or glutarate. Thus it would appear to be a C_6-OCH_3 MC. A possible structure is shown below, based on peaks at

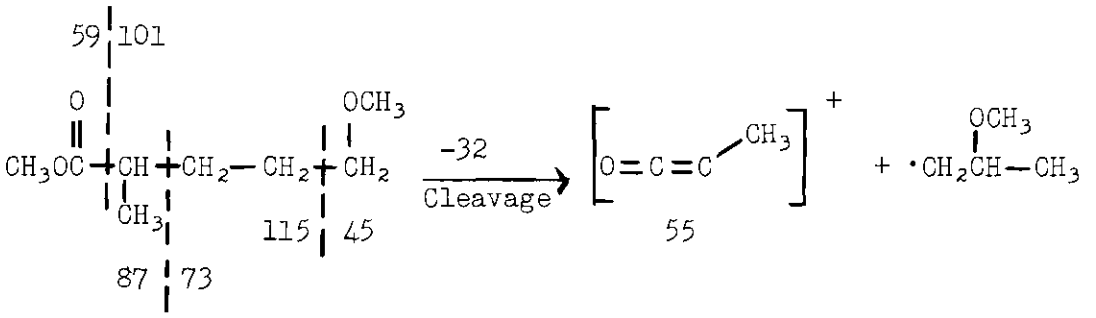


m/e 59, 70, and 101. However, the lack of a peak at m/e 145 is not consistent with this formulation. In addition, strong rearrangement peaks (i.e., m/e 74, 88, etc.) are not observed. No unique structure seemed to satisfy all these data.

Spectrum 6-10 appeared to be that of a C_8-MC, in that it showed a strong peak at m/e 127 (M-31) and a weak molecular ion peak at m/e 158. The lack of a strong rearrangement peak (m/e 74, 88, 102) indicated that

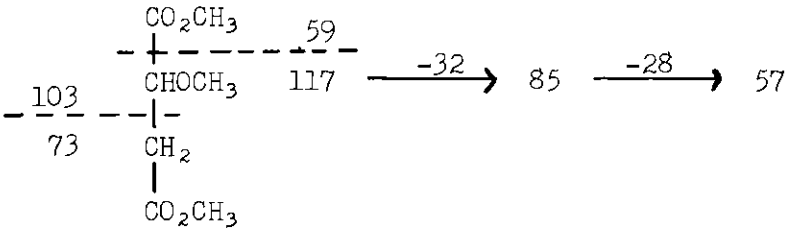
there was no hydrogen γ to the carbonyl group, or that if there was a γ hydrogen it was highly hindered.

Spectrum 7-10 was probably that of a C_6-OCH_3 MC because of the peaks at m/e 160(M^+), 129(M-31), 115(M-45), and 101(M-59). Very weak metastable peaks were observed at m/e 65.8 and 79.0, which corresponded to $115^+ \rightarrow 87^+ + 28$ ($M^* = 65.8$) and $129^+ \rightarrow 101^+ + 28$ ($M^* = 79.1$). The peak at m/e 88 was reasonably strong, indicating an α – CH_3 group. A possible structure is shown below. This structure accounts for all of the intense peaks and the observable metastable transitions.

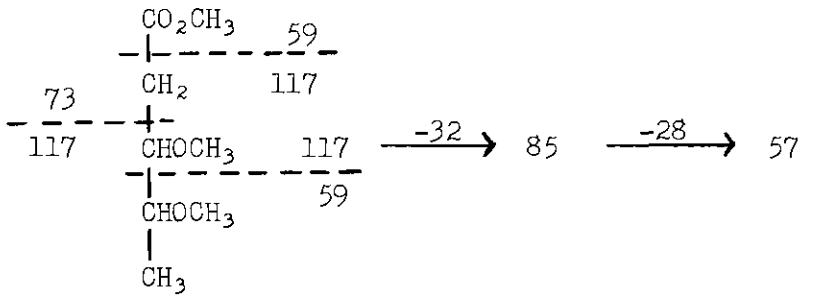


Spectrum 8-10 might be a C_5-DC because of the peak at m/e 129 (M-31). It was only somewhat consistent with glutarate and was not consistent with 2-methylsuccinate or dimethylmalonate. Of course, it is possible that it is a C_6-OCH_3 MC. An unambiguous structural assignment could not be made.

Spectrum 9-10 showed peaks at m/e 145 (M-31) and 117 (M-59) which were reasonable for a C_4-OCH_3 DC (MW = 176), or a C_5-di OCH_3 MC. The spectrum could be interpreted in terms of methoxysuccinate as shown below.

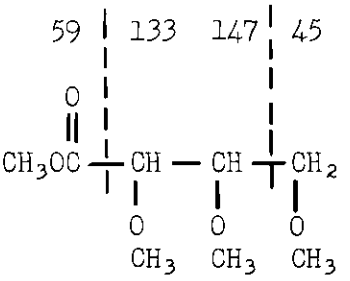


A similar interpretation could be rendered for the C_5-di OCH_3 MC.

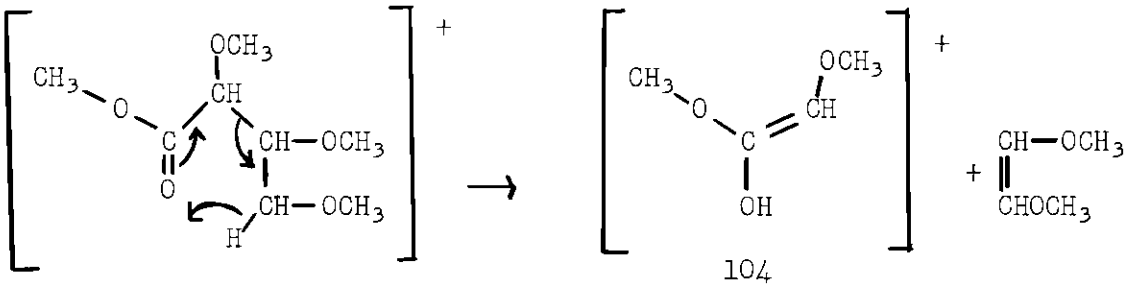


Spectrum 10-10 was somewhat similar to 9-10 in that it showed peaks at m/e 117, 85, and 59. There was a weak peak at m/e 174 (M^+) which was consistent with a C_7-OCH_3 MC or a C_6-DC. The components corresponding to 9-10 and 10-10 were not well resolved on this GLC column; both of the spectra probably arose from mixtures.

Spectrum 11-10 had a peak at m/e 161 as the most intense one in the high mass region. If this was an M-31 peak, the molecular weight would be 192. This is partially confirmed by the very intense peaks at m/e 133 and 59 (133 + 59 = 192). However, this molecular weight was not consistent with any mono or di-carboxylic methyl ester (and those containing one or two methoxyl groups). The only reasonable formula for molecular weight 192 is $C_8H_{16}O_5$ which would be a tri-methoxy monocarboxylate, as shown below. The peaks at m/e 85 and 117 may in part be due to contamination by previous components (9-10, 10-10) that were not

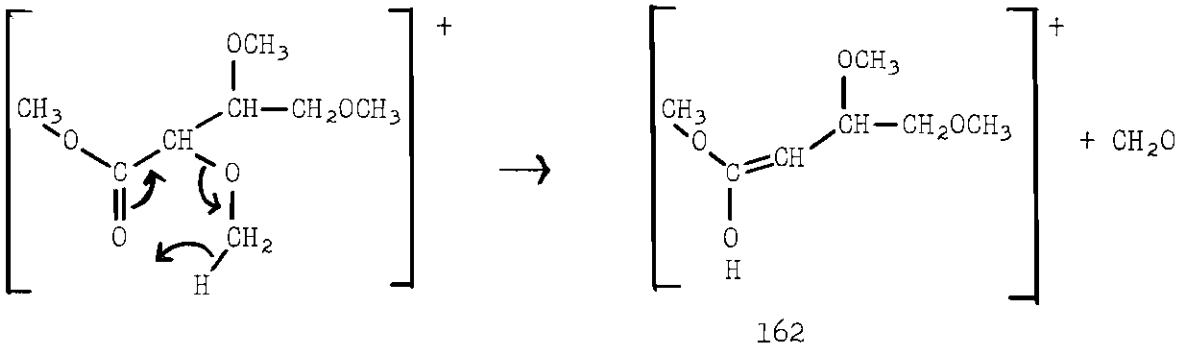


completely resolved. While this structure does not seem to be very attractive, it does seem to account for at least the most intense peaks. However, the lack of rearrangement peaks, as shown below, does not seem



104

reasonable. Another conceivable rearrangement would be:



162

But strong peaks at m/e 104 or 162 were not observed. Thus, the formulation of this component as a trimethoxy monocarboxylate did not seem reasonable. The peak at m/e 161 was therefore probably not a M-31 peak.

The GLC-MS data from the nitric acid oxidation of perhydroflaven-

somycin (low temperature) are summarized in Table 14. Spectra 1-14 and 2-14 did not appear to represent easily recognizable structures. Both spectra had peaks corresponding to benzene (m/e 78), and strong peaks at m/e 59. In addition, they both showed weak peaks at m/e 132, which is the molecular weight of malonate; however, neither was consistent with the published spectrum.

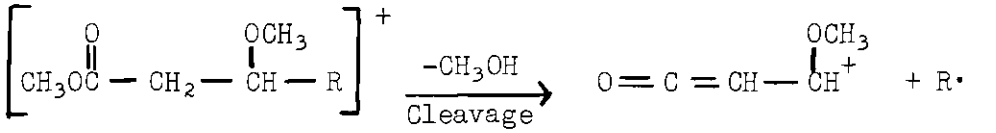
Spectrum 3-14 showed a weak peak at m/e 144, which corresponded to the molecular weight of hepanoate. It also had peaks at m/e 112 (M-32), 113 (M-31), and 115 (M-29), all of which are characteristic of methyl esters of monocarboxylic acids (56). The lack of any strong rearrangement peak indicated that there were no hydrogens γ to the carbonyl group (or very hindered). No unique structure seems obvious.

Spectrum 4-14 appeared to be perfectly consistent with succinate, and 5-14 was consistent with 2-methyl succinate. Spectrum No. 6-14 is very similar to 7-10 (refer to previous discussion).

Spectra 7-14 and 8-14 were in good agreement with those of glutarate and 2-methylglutarate, respectively. The next one, 9-14, has as its highest mass peak, m/e 157. This would correspond to the M-31 peak for a C_7-DC or a C_8-OCH_3 MC. It is not consistent with any of the standards, but could possibly be a mixture, such as 2,3-dimethylglutarate and adipate. However, the strong peak at m/e 129 and the lack of a peak at m/e 111 make this doubtful. A C_7 or C_8-OCH_3 MC (MW = 174 or 188) might explain the strong peak at m/e 129, corresponding to the loss of 45 (CH_3O-CH_2) or 59 (CH_3-CH-OCH_3) mass units.

Spectrum 10-14 was unusual in that it had a very strong peak at m/e 85. None of the standards has a strong peak at this m/e value. It

was thought that a peak at m/e 85 could arise from a β-methoxy ester, as shown below. However, the mass spectrum of β-methoxy adipate did not

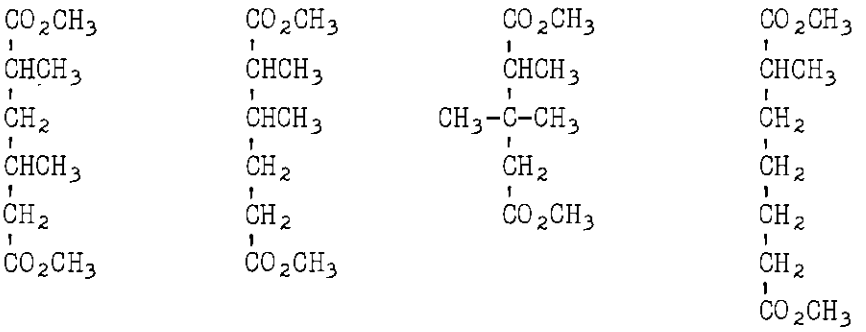


show a very intense peak at m/e 85 (relative intensity less than 10%). The nature of the ion of m/e 85 is therefore not clear at this time. The weak peak at m/e 171 (M-31) would correspond to a C_9-OCH_3 MC.

Spectrum 11-14 had a very strong peak at m/e 85. A C_6-OCH_3 DC was consistent with the peak at m/e 173 (M-31), and the strong peak at m/e 145 would be M-59.

Spectrum 12-14 was similar to 11-14 in that they both showed very strong peaks at m/e 85 and 145. The two components that these spectra represent were not well separated on this GLC column; probably both spectra were of mixtures of the two components.

Spectra 13-14 and 16-14 were very similar; the only real differences were small changes in the relative intensities. Component 16-14 was probably contaminated with component 17-14 (they were not well separated) thus accounting for the increased intensities of peaks at m/e 85 and 159 in spectrum 16-14 relative to spectrum 13-14. Both of these were probably C_8-DC, showing peaks at m/e 171 (M-31), 143 (M-59), and 142 (M-60). Neither of them was 3-ethyl-3-methylglutarate, 2,5-dimethyladipate, or suberate. The strong peaks at m/e 88 suggested α-CH_3 substitution, and the peak at m/e 74 showed that the other α position was unsubstituted. The four possible structures are shown below. It does not



appear possible to select unique structures for these two components until more standards are available. Since spectra 13-13 and 16-13 were so similar, it was possible that they represent diastereoisomers; if this was true, 2,3,3-trimethylglutarate and 2-methylpimelate would be eliminated from consideration.

Spectrum 14-14 might be of a C_6-OCH_3 DC because of peaks at m/e 173 (M-31), and 159 (M-45, CH_3O-CH_2). The weak peak at m/e 189 would correspond to a C_5-di-OCH_3 DC. Spectrum 15-14 was almost identical to 14-14, and since these components were not completely separated on this GLC column both spectra were probably of mixtures of these two components.

Spectra 17-14 and 18-14 were very similar, and may in fact both be of the same component since little, if any, separation was observed. The weak peak at m/e 187 (M-31) suggested that it may be a C_7-OCH_3 DC. Thus the peak at m/e 159 would be M-59, and the peak at m/e 129 would be M-91 (59 + 32, $\overset{O}{\overset{\|}{C}}OCH_3$ + CH_3OH).

Spectra 19-14 and 20-14 were almost identical and may be diastereoisomers. The peaks at m/e 74 and 88 suggested that one α-position was unsubstituted and the other was methyl substituted. These spectra were quite similar to 13-14 and 16-14 except that they appeared to be the next higher homologue (M-31 peak at m/e 185, thus a C_9-DC). As with spectra

13-14 and 16-14, no definite structure could be deduced until more standards have been determined.

Table 11 summarizes the GLC-MS data from the nitric acid oxidation of flavensomycin-high temperature. In total, 24 mass spectra were obtained. However, only the nine most intense components were studied in detail. Since these spectra were determined using a higher column temperature, the background was much higher. In addition, because of the complexity of this mixture it was very likely that many of the spectra were of mixtures of components that were not, or only partially, separated.

Spectrum 1-11 showed as the most intense high mass peak m/e 211, which corresponded to the M-31 peak for tetradecanoate. A weak peak was observed at 242 (M^+). However, the characteristic peak at M-43 (56) was only very weak, and is usually as strong as the M-31 peak. Other strong peaks in the spectrum were at m/e 55, 59, 69, 74, and 87, and are characteristic ester peaks. The strong peaks that were at m/e 84 and 98 are generally only observed dicarboxylic esters (56). The peak at m/e 213 would correspond to a C_{11}-DC. This spectrum appeared to be of a mixture of the C_{14}-MC and C_{11}-DC.

Spectrum 4-11 appeared to be a mixture of dodecanedioate with some other component. The strong peaks at m/e 74, 84, 98, 112, 153, and 185, in addition to the peak at 227 (M-31) were all in good agreement with those of the standard. The highest mass peak at m/e 273 (M-31) would correspond to a C_{11}-<u>di</u>-OCH_3 DC (MW 304) as a possible impurity.

Spectrum 5-11 showed as the highest mass peak, m/e 271. This would correspond to the M-31 peak for a C_{13}-OCH_3 DC. The rest of the spectrum did not appear to be consistent with this formulation. There

was a very noticeable lack of rearranged ions (even mass) of any great intensity. Cleavage peaks of the type $CH_3\overset{O}{\overset{\|}{O}}C-(CH_2)_n-\overset{OCH_3}{\overset{|}{CH}}^+$ were also not observed. This component, was therefore probably not a OCH_3 DC.

Spectrum 6-11 showed peaks at m/e 235 (M-31) and 266 (M^+) as the only prominent ones in the high mass region. This molecular weight did not correspond to any MC or DC (with or without methoxyl groups) that did not contain carbon-carbon double bonds and/or rings. A C_{16} MC with two rings would have a molecular weight of 266. Weak peaks at m/e 241 (M-31) and 98 (56) suggested that this component contained a small amount of tridecanedioate (MW = 272).

The peaks at m/e 270 (M^+), 241 (M-29), 239 (M-31), 227 (M-43), 143, 87, and 74, in spectrum 7-11 were all in good agreement for hexadecanoate (56). It was possible that there was a small amount of the C_{13}-DC in this component because of the increased intensities of peaks at m/e 84, 98, 112, and 241. The retention times for these two compounds were very similar on SE-30 columns.

Spectrum 10-11 was very interesting in that it showed what appeared to be a molecular ion at m/e 294 and characteristic strong peaks at m/e 263 (M-31), 262 (M-32), 235 (M-59), and 203 (M-32-59). A metastable peak was observed at 175.5 which corresponded to the transition $235^+ \rightarrow 203^+ + 32$ (M^* = 175.3). Since the elements of methanol were lost after m/e 59 (CO_2CH_3) was lost this component must have at least two CH_3O-groups (both could be $-CO_2CH_3$). The molecular weight of 294 did not correspond to any MC or DC (or those containing three or less methoxyl groups) that does not contain carbon-carbon double bonds and/or rings. For compounds containing more than three methoxyl groups (unlikely) a

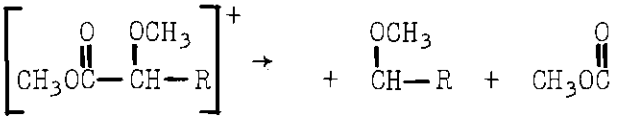
C_6-tetra OCH_3 DC or a C_7-penta OCH_3 MC both have molecular weights of 294. No DC or TC containing two or less rings and/or methoxyl groups would fit for this molecular weight (294). A C_{15}-DC containing three rings or a C_{18}-MC containing two rings has molecular weight 294.

Spectrum 13-11 showed a weak molecular ion at m/e 298, and other peaks at m/e 269 (M-29), 267 (M-31), 255 (M-43), 199, 143, 87, and 74 all of which are consistent for octadecanoate (stearate). The intensities of some of the peaks were not in perfect agreement with the standard but the overall fit was good.

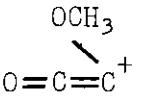
Spectrum 20-11 did not show any prominent peak in the high mass region. The strong peak at m/e 57 suggested the possibility of a methoxy ester (see previous discussion). The reasonably strong peaks at m/e 149 and 193 did not appear to correspond to any known type of cleavage peaks. This spectrum could not be easily rationalized in terms of the known fragmentation patterns for any simple MC or DC (and those containing methoxyl groups).

Spectrum 22-11 had as its most intense high mass peak m/e 279. The intense peaks at m/e 167 and 149 were related by the metastable peak at m/e 133 ($167^+ \rightarrow 149^+ + 18$, $M^* = 132.9$). The loss of 18 mass units corresponds to loss of water. The peak at m/e 279 was not consistent with M-31 (MW 310), as this did not correspond to any mono or di-carboxylate (with three or less methoxyl groups). Possible compounds, with molecular weights of 310, that contained two or less rings included: C_{19} MC (one ring), C_{16} DC (two rings), and C_{17}-OCH_3 MC (two rings). The ion at m/e 167 probably corresponded to $O{=}C{=}CH{-}(C_9H_{18})$, since the loss of 18 mass units to form an ion with m/e 149 is a known reaction (56).

Table 15 summarizes the GLC-MS data from the nitric acid oxidation of perhydroflavensomycin-high temperature. Spectrum 1-15 showed a weak peak at m/e 243 (M-31), which was consistent with a C_{11}-OCH_3 DC or a C_{12}-di OCH_3 MC. Other strong peaks were observed at m/e 219 (M-55), 215 (M-59), 183 (M-59-32), 151 (M-59-64), and 85. Some of these peaks were related by metastable transitions at 155.8 and ca. 125; $215^+ \rightarrow 183^+ + 32$, $m^* = 155.7$ and $183^+ \rightarrow 151^+ + 32$, $m^* = 124.7$. Since loss of 59 mass units (CO_2CH_3) was followed by two losses of 32 mass units (CH_3OH) it appeared as if this component was a C_{12}-di OCH_3 MC. However, there were no strong peaks observed for simple chain cleavage next to the methoxyl groups as is usually observed (56). Perhaps one of the methoxyl groups is close to the carboxyl group (α or β) thus allowing for very ready cleavage as shown below. The ion of m/e 71 might have the structure shown below.



M-59



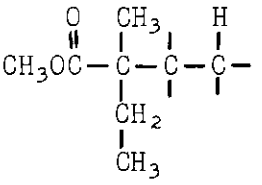
71

The quite strong peak at m/e 219 (M-55) is rather unusual for methyl esters. The possible position of another methoxyl group might have some relationship to this peak.

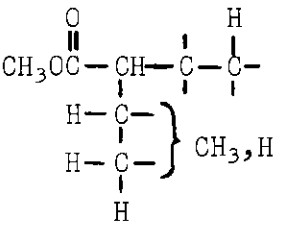
Spectrum 5-15 was consistent with hexadecanoate (see previous discussion) except for peaks at 247, 71, and 57 (prominent peaks) and a series of several other, less intense peaks. The spectrum thus appeared

to be of a mixture of components. The peak at 247 does not correspond to the M-31 or M-59 peak for any mono or dicarboxylate (and those containing two or less methoxyl groups).

Spectrum 7-15 was very interesting in that it showed very strong rearrangement peaks at m/e 116 and 230. The peak at m/e 116 would indicate one of the following part structures. If part structure XVIII is

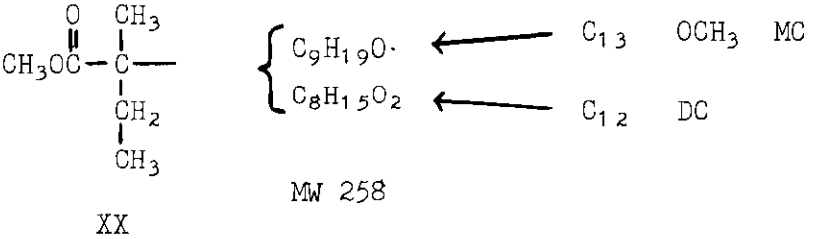


XVIII



XIX

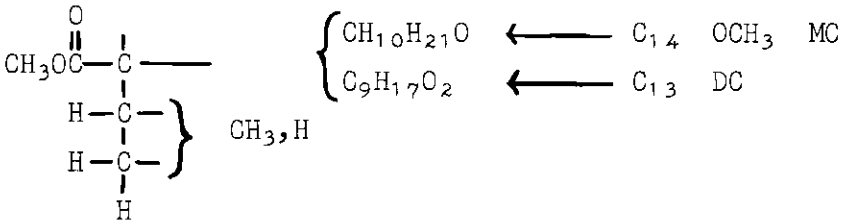
correct, then the other rearrangement peak at m/e 230 demands the following, if this component is a mono or dicarboxylate. Similarly, part



XX

MW 258

structure XIX would require the following.

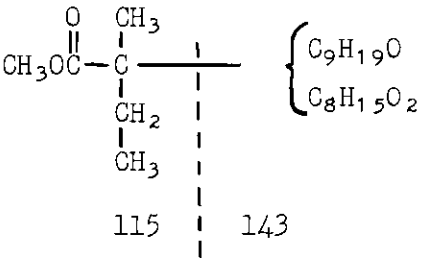


XXI

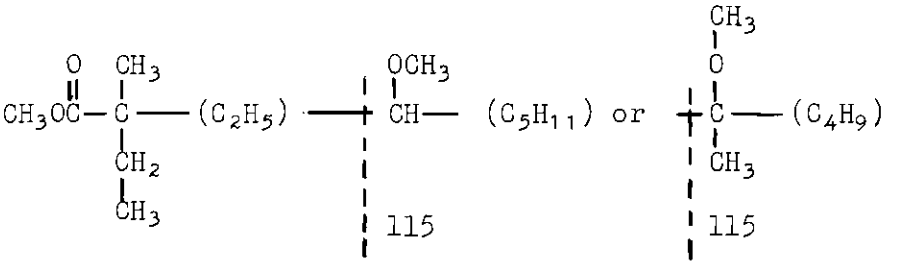
MW 272

The peaks at m/e 258 (M^+), 227 (M-31), 226 (M-32) and 198 (M-60) strongly support part structure XX, incorporating part structure XVIII. The relative intensities of peaks at m/e 272 (M^+) and 241 (M-31) were less than 0.5%.

Part structure XX would then represent either a C_{13}-OCH_3 MC or a C_{12}-DC. A decision between these two possibilities can be made on the basis of the base peak at m/e 115. Cleavage of the 2,3 bond would form an ion with m/e 115 as shown below. If the structure is a C_{13}-OCH_3 MC

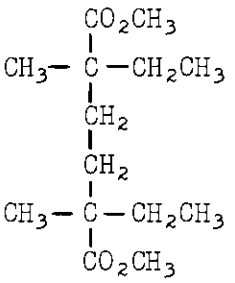


then cleavage next to the methoxyl group could also produce an ion with m/e 115 as shown below. Generally cleavage on both sides of the methoxyl

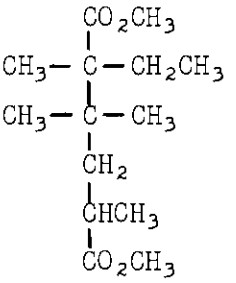


group is observed (56); the lack of strong peaks at m/e 187 ($M-C_5H_{11}$) or 201 ($M-C_4H_9$) indicates that this component is probably a C_{12}-DC.

No other strong rearrangement peaks were observed; this indicated that either the other carboxyl group had no hydrogen γ to it or that the molecule was symmetrical. Thus, the following two structures appear



XXII



XXIII

possible. It is possible that the number of γ hydrogens may, in part, determine which of two (or more) rearrangement peaks will be the most intense. Thus, for 2,3-dimethylglutarate the peak at m/e 74 (four γ hydrogens) and 88 (five γ hydrogens) have relative intensities 11 and 79, respectively. For 2-n-hexyltridecanoate (56), where there are equal numbers of γ hydrogens for the two rearrangements, the peaks have almost equal intensities (100 and 80). Structure XXII would thus predict that m/e 230 would be more intense than m/e 116 (three and two γ hydrogens, respectively). Structure XVIII would predict that m/e 116 would be more intense than m/e 230 (eight and three γ hydrogens, respectively). Although this type of reasoning does not have enough experimental data to make it a useful correlation, it does suggest that XXIII is the most likely structure for this component (the relative intensity of the peaks at m/e 116 and 230 are 98 and 44, respectively).

Spectrum 13-15 was perfectly consistent with that of octadecanoate (sterate).

Spectra 16-15 and 17-15 were very similar, and showed prominent peaks at m/e 215, 183, 151, 123, 95, and 81. Metastable peaks were observed at m/e 155.8 and 124.8, corresponding to the transitions $215^+ \rightarrow 183^+ + 32$ ($M^* = 155.8$) and $183^+ \rightarrow 151^+ + 32$ ($M^* = 124.5$). Spectrum 16-15

did not show any prominent peaks in the high mass region. Spectrum 17-15 showed a weak peak at m/e 299 (M-31) and a stronger one at m/e 239 (M-91; i.e., 31 + 60) (56) which were consistent with a C_{16}-di OCH_3 MC or a C_{15}-OCH_3 DC. The peak at M-91 is characteristic of a DC (56). Thus the strong peak at m/e 215 would correspond to a M-115 (M- CH_3OC-(C_4H_8)-) peak. The peaks at m/e 183 and 151 would correspond to two successive losses of methanol. It does not seem possible to derive more information from this spectrum because of the lack of rearrangement peaks and peaks due to other simple cleavage reactions.

Spectrum 19-15 showed weak peaks at m/e 229 (M-31) and 201 (M-59) which were consistent with a C_{10}-OCH_3 DC or a C_{11}-di OCH_3 MC. These assignments did not seem reasonable, because this component was eluted after octadecanoate (13-15); on the basis of molecular weight (boiling point) they should be eluted before octedecanoate. For example, hexadecanoate (MW = 270) and tridecanedioate (MW = 272) have almost identical retention times on SE-30. There was a very weak peak at m/e 313 (relative intensity ≈0.5%) which would correspond to the M-31 peak of a C_{16}-OCH_3 DC or a C_{17}-di OCH_3 MC (MW = 344). The peaks at m/e 229 and 201 would then be M-115 and M-143, respectively. The very strong peaks at m/e 187 (M-157) and 155 (M-157-32) were related by the metastable transition at m/e 128.5 ($187^+ \rightarrow 155^+ + 32$, $m^* = 128.4$). The peak at m/e 187 could correspond to $[CH_3O\text{-}(C_6H_{12})\text{-}CH^{OCH_3}]^+$ and m/e 155 to $[CH_3OC\text{-}(C_6H_{11})=CH]^+$. The lack of rearrangement peaks or other strong peaks in the high mass region made formulation of a possible structure very difficult.

Spectra 29-15, 31-15, 33-15, 34-15, and 35-15 were all quite similar. In general, they showed strong peaks at m/e 85, 95, 123, 127, 141,

145, 151, 155, 159, 173, 187, and 215. Metastable peaks at m/e 128.5, corresponding to the transition $187^+ \rightarrow 155^+ + 32$ ($m^* = 128.4$), were observed in all of these spectra. In spectra 29-15, 33-15, and 34-15 metastable peaks were observed at m/e 115 ($173^+ \rightarrow 141^+$ 32, $m^* = 115$). No prominent peaks were observed in the high mass regions (m/e > 300) of these spectra. Spectra 31-15, 33-15, and 34-15 showed very weak peaks at m/e 332, 374, and 388, respectively. These m/e values would correspond to M^+ ions for the C_{13}, C_{16}, and C_{17}-di OCH_3 DC, respectively. These spectra thus appeared to be of a series of dimethoxy dicarboxylates. Little structural information can be obtained from these spectra until the spectra of more standards become available.

Another mass spectral investigation was performed on the methyl esters derived from the nitric acid oxidation of flavensomycin and perhydroflavensomycin. The data from these runs are not given in this thesis. The samples used were derived using the same experimental procedure, but were from different reactions. The only significant differences between these runs, and the ones already discussed was that a 50-ft. GLC column was used, instead of a six-foot column (both 3% SE-30). The use of a longer column allowed much greater separation of the components. However, components with high boiling points had very long retention times on the 50-ft. column, and many of the higher molecular weight components were not observed. Since the data are not presented, only those components whose mass spectra gave excellent agreement with the standards will be enumerated.

Thus from the nitric acid oxidation of flavensomycin, the following compounds were identified: pimelate, suberate, azelaate, sebacate,

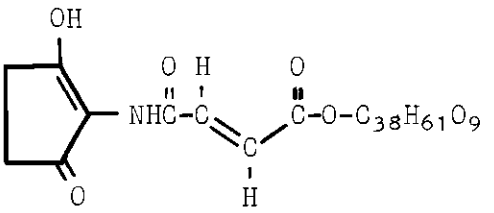
undecanedioate, and dodecanedioate (dodecanedioate was the last component whose mass spectrum was taken).

From the nitric acid oxidation of perhydroflavensomycin, the following compounds were identified: 2-methylsuccinate, 2-methylglutarate, and both isoners of 2,4-dimethylglutarate.

CHAPTER IV

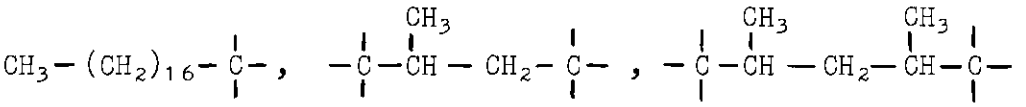
CONCLUSIONS

The structures that have been proposed (11) for flavensomycinoic acid (XVII), and its various derivatives have been confirmed. The pK_a of flavensomycin suggests that the attachment of the flavensomycinoic acid portion to the remaining part of the molecule is through the carboxyl group, which is also in agreement with the n.m.r. spectrum. This, taken with the proposed formula ($C_{47}H_{69}NO_{14}$) would lead to the following part structure for flavensomycin. The $C_{38}H_{61}O_9$ fragment must be

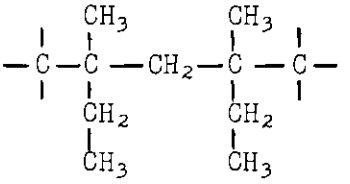


readily isomerized and/or degraded, since several mild reactions lead to complex mixtures of products. In addition, it must satisfy the following conditions: contain eight double bonds and/or rings; have six double bonds and/or rings that are easily reduced (platinum in acetic acid), a nonreducible carbonyl group, and hence a maximum of one ring; contain three $-OCH_3$ groups; have about 10 $C-CH_3$ groups; have about six to eight active hydrogens; contain at least one group that is capable of consuming base under saponification conditions. The products identified from nitric acid oxidation of flavensomycin indicate that the

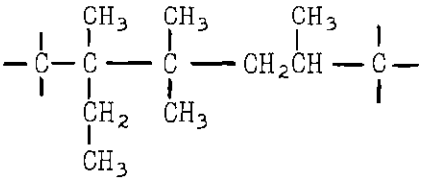
carbon skeleton of the $C_{38}H_{61}O_9$ fragment must satisfy the following part structures: $CH_3-(CH_2)_{16}-\overset{|}{C}-$, and $-\overset{|}{C}-(CH_2)_{10}-\overset{|}{C}-$. These two units are probably not independent of each other. When flavensomycin is fully reduced (perhydroflavensomycin) the carbon skeleton must satisfy the following part structures:



and tentatively either



or



Ozonolysis of flavensomycin, followed by reductive work-up, was shown to yield ethylene glycol and propylene glycol (58). Flavensomycin was shown not to react with sodium periodate (59). In addition, there was no evidence that a sugar component was released during attempted methanolysis of flavensomycin (59).

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CHAPTER I

INTRODUCTION

Nuclear Magnetic Resonance and Magnetic Nonequivalence

The first observations of the phenomenon of nuclear magnetic resonance (n.m.r.) in bulk matter were made independently in 1946 by Purcell, Torrey, and Pound (1) and by Block, Hansen, and Packard (2). Until about 1951 this technique was used entirely by physicists and physical chemists to study nuclear parameters. However, at this time, Gutowsky observed that the characteristic frequency at which protons absorb radiation was to some extent dependent upon their molecular environments (3). This behavior was referred to as the chemical shift. Thus it appeared that n.m.r. might have some possible application in the determination of molecular structure. This possibility was in fact very quickly realized. Great advances in both the experimental techniques and the theoretical aspects of n.m.r. soon followed. These advances have made it one of the most powerful techniques that the chemist may use.

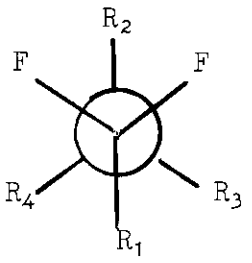
One of the areas of n.m.r. research has dealt with the determination of the nature of the chemical shift difference which is sometimes observed between atoms or groups that otherwise appear to be equivalent.

In 1956 Drysdale and Phillips (4) noted that the geminal fluorine atoms in compounds of the type $R_1F_2C-CR_2R_3R_4$ (I) (R_1 = Br, Cl; R_2 = H, F; R_3 = Cl, Br; R_4 = Br, Ø) were magnetically nonequivalent (had different chemical shifts) even though it was expected that rapid rotation about

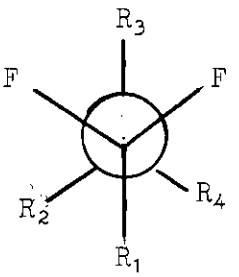
the carbon-carbon bond would average their magnetic environments. Thus, the authors concluded that there was restricted rotation about the carbon-carbon bond and that they were actually observing the n.m.r. spectrum of only one of the rotamers. Temperature studies of the n.m.r. spectra of these compounds (up to 200°) revealed that in no cases did the spectra collapse to simpler ones (although in one case the chemical shift difference, $\Delta\nu$, changed markedly with temperature). Thus it appeared that rotation was not rapid even at the upper temperature limit.

This behavior suggested (5) that compounds of the type R_1CF_2-$CR_3R_3R_2$ (II) (R_1 = Br; R_2 = F, Br, Ø; R_3 = Br, Cl, Br) might be capable of optical resolution at room temperature, since the barriers for rotation might be large. However, this expectation was not realized.

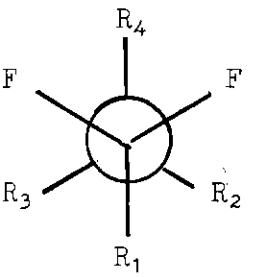
In fact, the n.m.r. spectra of these compounds showed that the geminal fluorines were equivalent at all temperatures down to -30°. These results indicated that there was rapid rotation about the carbon-carbon bond, even at room temperature and below. The solution suggested to this apparently paradoxical situation was: "the chemical shift between gem-groups as in compounds like I is not necessarily averaged by rapid rotation unless the residence times of the molecule in each of the various rotation conformation (Ia, Ib, Ic) are equal (5)." It was pointed out that geminal fluorine atoms are not equivalent in any of the three conformations Ia, Ib, or Ic, and thus $\Delta\nu$ will not have an average value in the general case. For compounds of the type II, only two of the three conformations, namely IIb and IIc have nonequivalent geminal fluorine atoms. However, the conformers must have equal populations since they are merely mirror images. The third conformer, IIa, may have quite a different



Ia

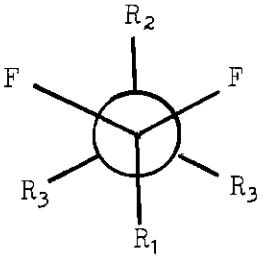


Ib

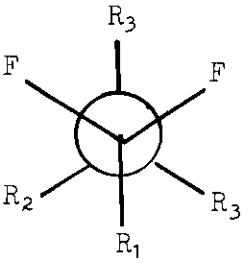


Ic

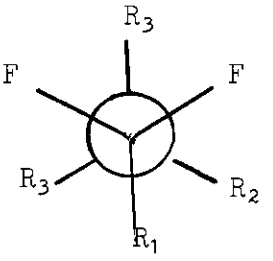
population but the geminal fluorines are equivalent. However, at -80°, rotation about the carbon-carbon bond was slow enough to observe the different conformers in the case where R_1 = Br, R_2 = Br, and R_3 = Cl (5).



IIa



IIb



IIc

These authors (5) also studied the proton n.m.r. spectrum of methyl 2,3-dibromo-2-methylpropanoate. The geminal protons were also nonequivalent in this compound. The geminal protons in 1,2-dibromo-2-methylpropane were, however, equivalent. From the above data, the following conclusions were reached:

1) The residence times and populations of the various conformers (as Ia, Ib, and Ic) are not equal.

2) In principle it is possible to estimate, or perhaps determine, the population of the various conformers.

3) The populations of conformers, in favorable cases, may be

determined directly, by "freezing in" at low temperature.

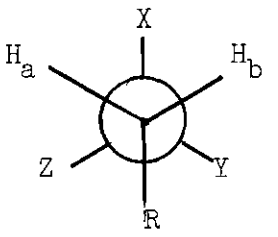
4) A new, and simple means is provided for possible detection of carbon atoms which do not possess a plane of symmetry, without recourse to optical resolution (they point out that 2-methyl-2-nitropropane-1,3-diol shows nonequivalent geminal hydrogens, although it is not capable of resolution; in this case each of the methylene groups is attached to a group which has no plane of symmetry, although the molecule as a whole possesses a plane of symmetry).

The explanations advanced above for the nonequivalence of geminal atoms (or groups) was referred to as the "time-weighted-average" postulate (6).

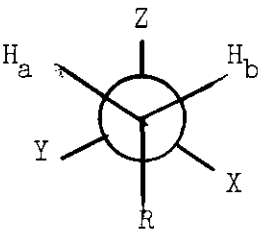
This type of nonequivalence of geminal protons was soon observed by several other workers (6, 7, 8, 9, 10). Finegold (7,8) noted that the methylene protons in $CH_3CH_2O\overset{O}{\overset{\|}{S}}O-CH_2CH_3$ (III) and $CH_3\overset{S}{\overset{\|}{P}}-(OCH_2CH_3)_2$ (IV) did not give "normal" patterns, but appeared as two multiplets, having different chemical shifts. Finegold proposed that the two $-O-CH_2CH_3$ groups were nonequivalent, presumably resulting from different S-O, or P-O bond orders. This explanation was, however, shown to be incorrect (9,10). The bonds to the sulfur or phosphorus atoms in III and IV are not planar, and thus the methylene protons experience the effect of this lack of symmetry. In addition, a more careful examination of the n.m.r. spectra of these compounds revealed that the nonequivalent protons were strongly coupled to each other (ca. 10 cps). This would only be expected if the nonequivalent protons were attached to the same atom and not separated by six bonds. In addition, diethyl sulfoxide, ethyl isopropyl sulfoxide, diethylmethyl ammonium iodide, and the diethyl sulfide-borane adduct

showed nonequivalent methylene protons,* whereas diethyl sulfate, diethyl sulfone, and the diethyl sulfide-boron trifluoride adduct* did not show this nonequivalence (11). Diethyl carbonate does not show nonequivalent methylene proton (7), whereas isopropylphenylphosphinyl chloride and its methyl ester both show nonequivalent geminal methyl groups (12).

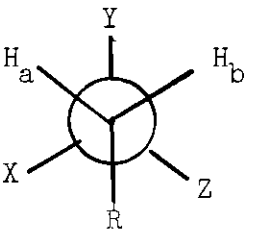
Pople (13), Waught and Cotton (9), Jackman (14), and Coyle and Stone (11) pointed out some important consideration which the "time-weighted-average" postulate had overlooked. They proposed the theory of "intrinsic asymmetry." In principle, nonequivalence can still exist when internal rotation is free and when the rotamers are all accidently of equal energy. This can easily be seen by inspecting the three rotamers Va, Vb, and Vc. In any given conformation (i.e., Va) the environment of



Va



Vb



Vc

H_a is different from H_b and in no other conformation (Vb, Vc) is the environment of H_b equal to that of H_a in conformation Va. In the general case H_a will never be equivalent to H_b (except by chance). It has been pointed out that "it seems unlikely that in practice the asymmetry with respect to internal rotation required to produce magnetic nonequivalence

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* It was suggested that exchange of the BF_3 group between the two lone-pair electron positions is responsible for the observed equivalence.

would not also be reflected in some angular dependence of potential energy" (9). The n.m.r. spectrum of acetaldehyde diethyl acetal was analyzed (9), and showed nonequivalent methylene hydrogens ($\Delta\nu$ = 0.152 ppm, J_{gem} = 9.2 cps and J_{vic} = 6.7 and 7.2 cps).

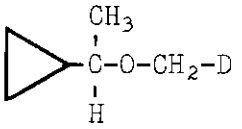
Other authors noted that ethyl groups next to a center of asymmetry did not give simple patterns (10). Analyses of the spectra of acetaldehyde diethyl acetal and α-cyclopropyldiethyl ether gave values of $\Delta\nu$ = 0.15 ppm, J_{gem} = 9.4, J_{vic} = 7.35 and 6.68 cps; $\Delta\nu$ = 9.0 cps, J_{gem} = 0.157 ppm and J_{vic} = 7.35 and 6.68 cps, respectively. Since each of these compounds appeared to have <u>two different vicinal</u> coupling constants, it was thought that asymmetry was being induced in the bonding of the methylene protons to carbon. The fact that there were no significant changes in the spectrum of acetaldehyde diethyl acetal upon cooling to -80° was interpreted in terms of one conformation being very strongly favored over this temperature range.

Roberts (15) later found that the n.m.r. spectrum of diethyl sulfite (50% in benzene) could be analyzed using either of <u>two sets</u> of parameters. Either $\Delta\nu$ = 5.77 cps, J_{gem} = 10.40 cps, J_{vic} = 7.69 and 6.60 cps or $\Delta\nu$ = 5.97 cps, J_{gem} = $\mp$ 10.45 cps, J_{vic} = $\pm$ 7.12 and $\pm$ 7.12 cps. In addition, he found that the spectrum of neat diethyl sulfite could <u>only</u> be analyzed when J_{gem} was opposite in sign from J_{vic}.

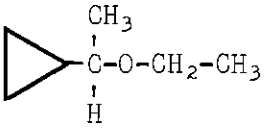
Theoretical calculations of <u>geminal</u> coupling constants (16) and <u>vicinal</u> coupling constants (17) had indicated that they should have the same sign (positive). Since the calculations of <u>vicinal</u> coupling constants seemed to be much more reliable (18) it appears that the theoretical treatment of <u>geminal</u> coupling constants was in error. This was confirmed by

many authors (18, 19, 20, 21, 22, 23) using the exact analysis technique (sometimes at a different field strength) and the double irradiation technique. It appears to be well established that for sp^3 hybridized carbon, geminal coupling constants are opposite in sign from vicinal coupling constants and are negative (24). Thus for acetaldehyde diethyl acetal, the alternate parameters $\Delta\nu$ = 9.23 cps, J_{gem} = -9.30 cps, and J_{vic} = + 7.03 cps appear to be correct (15).

Roberts et al. (25) have attempted to show that conformational effects, and not intrinsic asymmetry, are the most important factors that govern the nonequivalence of methylene protons. They studied the n.m.r. spectrum of cyclopropylmethylcarbinyl methyl-d_1 ether (VI) and compared it to the n.m.r. spectrum of cyclopropylmethylcarbinyl ethyl ether (VII). It would be expected that substitution of deuterium for methyl in the



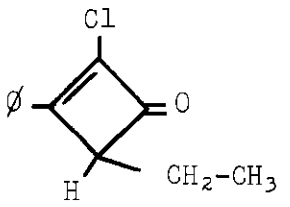
VI



VII

fragment $-O-CH_2-$ would eliminate any conformational preference of the methylene protons with respect to the asymmetric center. Thus, if any nonequivalence was observed, it must be due to the intrinsic asymmetry of the molecule. In fact, no chemical shift difference was observed for the methylene protons of VI, whereas in VII, $\Delta\nu \cong 9$ cps. This was interpreted as indicating that the chemical shift difference observed in VII is a result of some conformational preference. Further support for this viewpoint was offered by the n.m.r. spectrum of the cyclobutenone VIII.

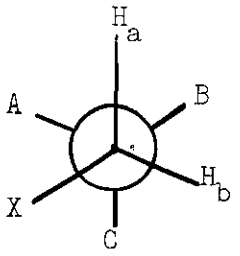
The signal for the methine proton is split into two doublets (J ≈ 4 and



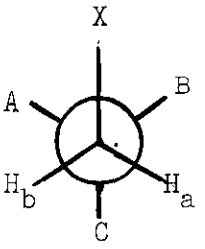
VIII

7 cps). The unequal coupling constants were explained as arising from a conformational preference for this molecule.

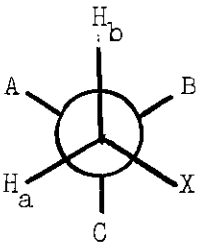
However, these conclusions were sharply criticized by Gutowsky (26). He presented a mathematical formulation in which the various effects could be separated. For a CH_2-X group in an asymmetric environment, the three rotational isomers are 1, 2, and 3. The rotationally averaged



1



2



3

chemical shifts $\langle \nu \rangle$ are given by

$$\langle \nu_a \rangle = \sum_n X_n \nu_n^a \text{ and } \langle \nu_b \rangle = \sum_n X_n \nu_n^b \qquad (1)$$

where X_n is the mole fraction of rotamer n, and ν_n^i is the chemical shift of the ith nucleus in rotamer n.

In the case where X = H, $\langle \nu_a \rangle = \langle \nu_b \rangle$ because of the symmetry of the CH_3 group (i.e., $\nu_1^a = \nu_3^b$, $\nu_2^a = \nu_1^b$, and $\nu_3^a = \nu_2^b$, and $n_1 = n_2 = n_3$). However, this tells nothing about the relative values of ν_1^i, ν_2^i, and ν_3^i, which reflect the intrinsic asymmetry of the three nonequivalent sites. These values could be determined if the rotational isomers could be "frozen out."

In the case where $X \neq H$, the energies (and thus populations) of the three rotamers will differ as will the six chemical shifts ν_n^a and ν_n^b. Thus the net chemical shift between H_a and H_b will be

$$\langle \nu_a - \nu_b \rangle = \sum_n X_n \, (\nu_n^a - \nu_n^b) \qquad (2)$$

which can be written as

$$\langle \nu_a - \nu_b \rangle = \sum_n X_n \, (\Delta\nu_n^{\,o} + \delta\nu_n), \qquad (3)$$

where $\Delta\nu_n^{\,o}$ is $(\nu_n^a - \nu_n^b)$ for the compound X = H, and $\delta\nu_n$ is the change in $\Delta\nu_n$ caused by the substitution of X for H.

Equation 3 may be written as

$$\langle \nu_a - \nu_b \rangle = \sum_n (X_n - 1/3) \, \Delta\nu_n^{\,o} + \sum_n (X_n - 1/3) \, \delta\nu_n + \sum_n 1/3 \, \delta\nu_n \qquad (4)$$

by including the fact that $\sum_n 1/3 \, \Delta\nu_n^{\,o} = 0$, and rearrangement. The first term depends on the population differences caused by $X \neq H$ and the intrinsic asymmetry of the parent compound (X = H). The second term depends upon both population differences and the change in chemical shifts caused by having $X \neq H$. The third term, however, depends only on the asymmetry effect caused by the introduction of X; thus this term is the

net chemical shift difference caused by X in the event the conformer populations were equal.

Gutowsky applied this equation to the compounds studied by Roberts (VI and VII). For VI, X = D and for VII, X = CH_3. In VI, if it is assumed that the conformations are equally populated, then $\sum_n \delta\nu_n = 0$; i.e., there is no asymmetry effect if $\langle \nu_a - \nu_b \rangle = 0$ (as observed). This was not unexpected in view of the small differences between D and H (27). Roberts' interpretation would have been correct if it could have been independently shown that deuterium substitution could cause an asymmetry effect rather than a conformational effect. This would, however, imply that the asymmetry effect was more important than the conformational effect, the opposite of what the experiment was intended to show. Thus Gutowsky concluded that the observed equivalence of the methylene protons in VI not only says nothing about the relative importance of conformational preference versus the asymmetry effect, but also it had no bearing upon the nonequivalence of the methylene protons in VII. He did propose a method to evaluate the relative importance of the various effects, namely the temperature dependence of $\langle \Delta\nu_H \rangle$ and its high temperature limit. At high enough temperature, the rotamers should be equally populated (28), and the observed nonequivalence is then $\sum_n 1/3\ \delta\nu_n$, the asymmetry effect. Gutowsky was able, by studying the temperature dependence of $\langle \nu_a^F - \nu_b^F \rangle$ for $CF_aF_bBrCFBrCl$, to estimate the chemical shift difference of the three rotamers (28); they were 178, 75, and -273 cps (at 40 Mc, $CFCl_3$ as the reference). The calculated value of the asymmetry effect, 6.7 cps, is to be compared with the rotationally averaged shifts of 84.75 to 46.45 cps over the temperature range -49 to 193°. Although the effect was rather

small, and there were considerable uncertainties in obtaining its numerical value, it seems probable that an asymmetry effect was present. It was also possible that intermolecular interactions contribute to the temperature dependence of $\langle \Delta\nu_F \rangle$.

It should be noted that the parameter $\delta\nu_n$ contributes to the rotationally averaged chemical shift *via* the second term in Equation 4. Thus equation 4 did not give separate values for $\Delta\nu_n^0$ and $\delta\nu_n$. However, several methods were proposed for obtaining values of $\Delta\nu_n^0$.

Snyder (29) reviewed the subject of nonequivalence of *geminal* groups and gave clear and concise definitions of the various terms that have been used. There are basically two means of describing the nonequivalence of nuclei; these are the chemical shift difference between them and the spin coupling constant of these nuclei with some other nucleus. For example, in the compound $CF_2=CH_2$, the two chemically equivalent hydrogens (or fluorines) are magnetically equivalent in the chemical shift sense, but are nonequivalent in the spin coupling sense (*i.e.*, $J_{trans} \neq J_{cis}$). However, it should be clearly recognized that the atoms (or groups) R_1 and R_2 in compounds of the type A-CR_1R_2-CEDF, "are *always stereochemically nonequivalent but not necessarily magnetically nonequivalent*." Mislow has suggested (30) that the term "diastereomeric" be used to refer to these atoms or groups to rigorously describe the stereochemical attribute.

Snyder (29) studied a series of compounds with diastereomeric protons or methyl groups and the olefins from which they were formally derived (*e.g.* $CH_2Br-\overset{CH_3}{\overset{|}{C}}BrCO_2H$ and $CH_2=\overset{CH_3}{\overset{|}{C}}-CO_2H$). It was observed that there was a definite correlation between $\Delta\nu$ in the saturated compound and $\Delta\nu$ in the corresponding olefin. He found that the differences in chemical

shift between nonequivalent hydrogens to be quite solvent dependent. This solvent dependence was also related to the dielectric constant of the medium; the higher chemical shift difference was associated with the solvent of lower dielectric constant. This type of behavior could easily be explained by changes in the conformational population. Since the olefin is a relatively fixed system, one would not expect any large changes in geometry with different solvents, whereas in the saturated compounds a change in solvent might result in a different population of conformers. Thus Snyder concludes that "the chemical shift between nonequivalent hydrogens in methylene need not accurately reflect changes in rotational equilibrium, but instead may merely reflect those magnetic properties of the solvent which affect chemical shift differences between hydrogens of a vinylic terminal methylene group." It was also observed that there was a relationship between $\Delta\nu$ and the mean resonance position of the methylene protons, $\bar{\nu}$. Thus the correspondence between $\Delta\nu$ and $\bar{\nu}$ was as good as that of $\Delta\nu$ of the saturated compound and $\Delta\nu$ of the olefin. Thus it seemed possible to explain the variation in $\Delta\nu$ in terms of the properties of the solvent that are related to local magnetic field anisotropy.

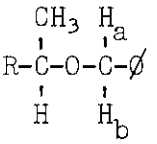
It is also interesting to note that $\Delta\nu$ for the methyl groups was larger than $\Delta\nu$ for the methylene proton in the compounds $\text{Ø-CHBr-C-(R)}_2\text{Br}$ (R = H or CH_3). For the pair of compounds $BrCH_2\text{-}CH_2\text{-}Br$ and $Br(CH_3)_2C\text{-}C(CH_3)_2Br$, the substitution of methyls for hydrogen decreased the differences in conformer population. If this holds for the former pair of compounds, then it is hard to rationalize the chemical shift difference in terms of conformer population. Snyder suggested that probably the most important effect was the magnetic anisotropy about the carbon bearing

the nonequivalent atoms or groups. It was strongly emphasized that chemical shift data are an inadequate measure of conformational equilibria.

Several other authors have observed the nonequivalence of geminal atoms or groups. Although few of these authors have specifically studied the problem in detail, most of them tended to agree with the "time-weighted-average," or conformational preference theory (12,31,32,33,34,35), but some thought that intricate asymmetry was the most important factor (36,37).

Roberts et al. (38,39) have studied this problem in detail. They point out (38) that the question is not whether conformational preference or intrinsic asymmetry is solely responsible for magnetic nonequivalence, but how much, if any, can be ascribed to intrinsic asymmetry. They state that "the existence of preferred conformations of the methylene group or the isopropyl group with respect to the asymmetric center has generally been considered necessary for magnetic nonequivalence...." and "there seems little doubt that conformational preference with respect to the asymmetric center must in general be responsible for the major contribitions to the magnetic nonequivalence."

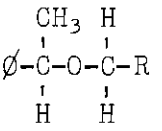
In general they studied compounds of the type IX that had different R groups. There seemed to be a correlation between the size of the



IX

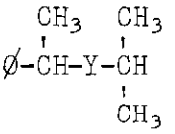
R group and $\Delta\nu_{ab}$. Thus, in carbon tetrachloride solution $\Delta\nu_{ab}$ (for different R groups) increased in the order ethyl < isopropyl < cyclohexyl

phenyl<t-butyl < carbethoxy. However, this order was not strictly followed in all solvents (e.g. in acetone $\Delta\nu_{ab}$ for R = Ø was the smallest); in addition, the carbethoxy group is not even larger than an ethyl group. However, for compounds of the type X where R was changed from methyl, isopropyl, t-butyl, and phenyl, the observed $\Delta\nu$ does not seem to follow this pattern.



X

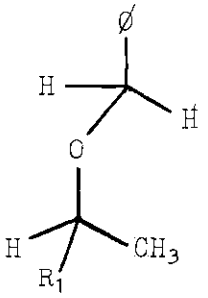
They also studied how the magnetic nonequivalence of isopropyl methyl groups varied with its proximity to the asymmetric center. Thus for compounds of the type XI having Y = -, -O-, $-OCH_2-$, $-OCH_2CH_2-$,



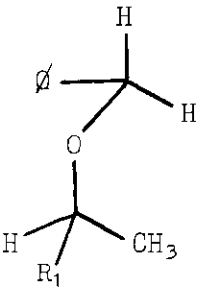
XI

$-OCH_2CH_2O-$, and $-OCH_2CH_2OCH_2-$, $\Delta\nu$ of the methyl groups in carbon tetrachloride changed in the order 11.7, 5.8, 0.0, 2.2, 0.0, and 0.0 cps, respectively. This same order was observed in other solvents. They also studied the solvent dependence of compounds of type IX where R is an alkyl group. The values of $\Delta\nu$ for a given compound varied by less than 1.5 cps over the range of solvents carbon tetrachloride, benzene, cyclohexane, acetone, dimethyl sulfoxide, acetic acid, and t-butyl alcohol. Thus it appeared that such factors as hydrogen bonding to the ether oxygen,

magnetic anisotropy of the solvent molecules, and dielectric constant of the solvent were not important in determining the extent of magnetic nonequivalence. Roberts proposed that XIIa and XIIb would represent the most populated conformations of compounds of type IX, and XIIa would be the most favored for the case in which R_1 was larger than methyl. He discounts the effects of the anisotropy of carbon-hydrogen and carbon-carbon single bonds as being an important contribution to the observed nonequivalence. Thus, if these effects were important, replacing the phenyl group with an isopropyl group should make relatively little change in the chemical shift difference. These groups have a similar size and were not expected to make a large conformational change. However, the data showed



XIIa



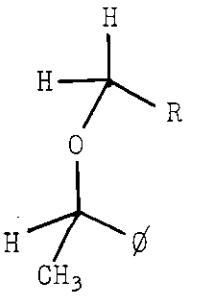
XIIb

that replacement of phenyl by isopropyl (on the $-OCH_2-$ group) did result in a large decrease in the observed magnetic nonequivalence. Roberts concluded that the origin of the magnetic nonequivalence was not primarily due to the magnetic anisotropy of the α-bonds.

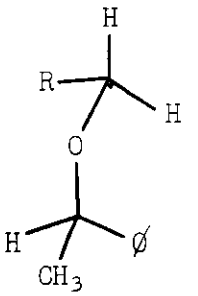
Roberts was able to calculate, using the method of Johnson and Bovey (40), that the maximum chemical shift difference between the methylene protons due to the directly bonded phenyl group was about 9 cps. This occurred when one proton was in the same plane as the benzene ring.

This is about the magnitude of $\Delta\nu$ which was commonly observed in these compounds.

In conformation XIIa, if R = phenyl, the maximum effect of the distant phenyl ring would be less than 1 cps. However, in other conformations such as XIIIa and XIIIb, the maximum effect of the phenyl group was estimated to be 3 and 30 cps, respectively. Roberts stated that there was no indication that conformer XIIIb was significantly populated in 1-phenylethyl benzyl ether (R = Ø), but that conformations of this type could possibly explain the large value of $\Delta\nu$ in the case where R = CO_2Et.



XIIIa



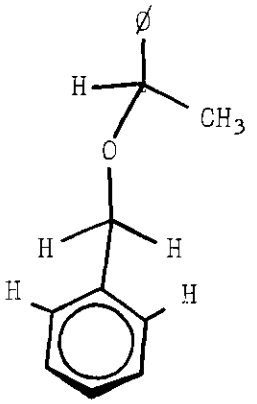
XIIIb

Roberts concluded that the conformation of the directly bonded phenyl group with respect to the methylene hydrogens was the most important factor in determining magnetic nonequivalence. This explanation could not account for the nonequivalence of the methyl groups observed in compounds of type XI. The fact that the values for $\Delta\nu$ did not fall monotonically to zero (they had a particularly large value when the methyl groups were separated by five bonds from the asymmetric center) suggested that possible coiled conformations were important in this case.

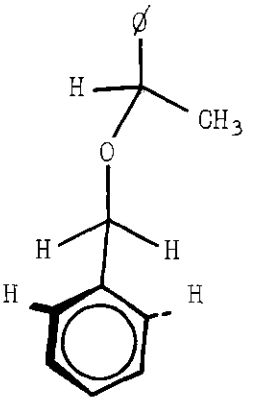
Roberts *et al.* (39) have also studied the solvent dependence of $\Delta\nu$ in 1-phenylethyl benzyl ether (XIV) in a wide variety of solvents.

They found that in general $\Delta\nu$ was inversely related to the dielectric constant of the solvent (notable exceptions were acetic acid, ethanol, formic acid, and dioxane). This was in contrast to the behavior of compounds of type IX where R was an alkyl group. They found that whenever R was an unsaturated group there was generally a large solvent dependence of $\Delta\nu$.

The observed solvent dependence of $\Delta\nu$ was explained by assuming that the solvent can alter the apparent "shape" of the asymmetric center by interaction with an unsaturated group. This could then change the configuration of the directly bonded phenyl group, possibly favoring (as extremes) either conformation XVa or XVb.



XVa

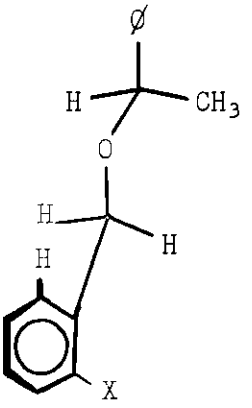


XVb

Support for this suggestion was offered by the apparent lack of a solvent dependence of $\Delta\nu$ in 1-indyl benzyl ether, in which the configuration of the benzene ring at the asymmetric center is fixed. The $\Delta\nu$ was too small to be observed in <u>all</u> of the solvents that were studied. Additional support for the idea that the configuration of the directly bonded phenyl group is the most important factor in determining magnetic

nonequivalence in these systems was obtained by studying a series of substituted 1-phenylethyl benzyl ethers. When the $-CH_2-\emptyset$ was substituted with a meta or para chlorine atom there were no significant differences in $\Delta\nu$ as compared to the parent compound. However, when the substituent was an ortho chlorine or bromine atom, no magnetic nonequivalence could be observed in any solvent. When the $-CH_2-\emptyset$ group was di-ortho substituted with chlorine atoms, $\Delta\nu$ was approximately the same as for the parent compound.

These results can be explained if a conformation such as XVI be-



XVI

comes important for the mono-ortho substituted ethers (X = Cl, Br). This type of conformation would minimize direct dipole-dipole interaction between the chlorine or bromine and the ether oxygen. When the phenyl group is di-ortho substituted, then presumably this type of conformation would not be favored, and a conformation analogous to XVa or XVb would be more populated.

Snyder (41) has recently studied a series of compounds of the

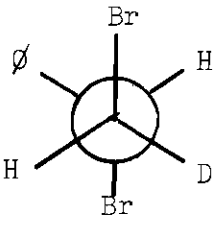
type $R_1R_2CH\text{-}CH_2\text{-}R_3$ (XVI) ($R_1 \neq R_2$). Exact analyses of the n.m.r. spectra confirmed that J_{gem} was opposite in sign from J_{vic}. In general, he was able to observe two different vicinal coupling constants, as Roberts had reported earlier (25). However, he does not feel that this is valid evidence against the intrinsic asymmetry theory.

It was observed that the vicinal coupling constants in compounds of type XVI showed a small, but real solvent dependence (the maximum change in J_{vic} was ca. 0.5 cps). Since it is generally thought that the magnitude of vicinal coupling constants is closely associated with the dihedral angle between the two protons, this behavior could reflect changes in the conformational equilibrium. In contrast to the small solvent dependence of J_{vic} was the large solvent dependence of $\Delta\nu$ between the nonequivalent methylene proton (the maximum change in $\Delta\nu$ was ca. 25 cps). These results indicated that chemical shift data, per se, are not good criteria for deriving data about conformer population. Snyder does point out that this result is not in conflict with Roberts (39), since the conformational changes that Roberts considers are quite different than in this work.

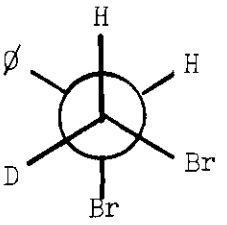
Analysis of the n.m.r. spectra of $BrCH_2\text{-}CHBr\emptyset$ in various solvents suggests the interesting possibility that a "cross-over" in chemical shifts had occurred (i.e., the proton absorbing at lower field in one solvent became the proton absorbing at higher field in another solvent). This was unequivocally proved by specific deuterium labeling (42). This again pointed to the inadequcy of chemical shift data in regard to conformational equilibrium.

Snyder was able to show, by this specific deuterium labeling,

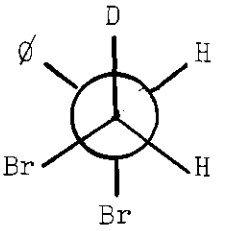
which of the conformers of two tri-substituted ethanes were most populated at room temperature. He was able to synthesize erythro-Ø-CHBr-CHDBr (XVII) and threo-$(CH_3)_3C$-CHBr-CHDBr (XVIII). The n.m.r. spectrum of XVII



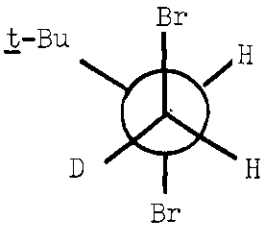
XVIIa



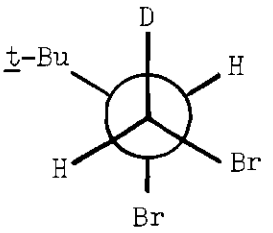
XVIIb



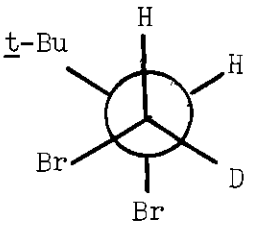
XVIIc



XVIIIa



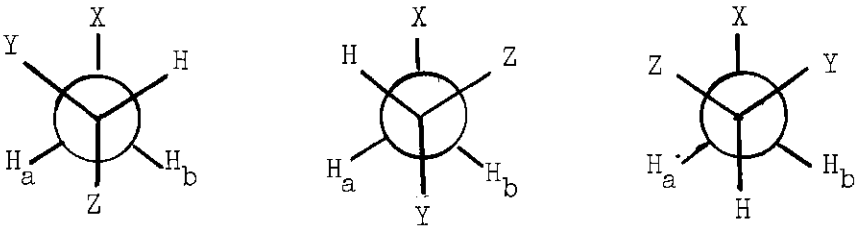
XVIIIb



XVIIIc

showed that J_{HH} was 10.5-11.0 cps, whereas in XVIII, J_{HH} was 9.2-10.1 cps. It is generally accepted that vicinal trans coupling constants in freely rotating ethanes are of the order of 10-17 cps and gauche coupling constants are not even as high as 6 cps. This would indicate that XVIIa and XVIIIb (i.e., the conformers with trans hydrogens) were the most populated conformers. It is interesting to note that in XVIIa the two bromine atoms are trans, whereas in XVIIIb, the t-butyl group is trans to the bromine atom. Snyder proposed that it was possible that an attractive force between gauche phenyl-bromine stabilized conformer XVIIa relative to SVIIb since he was not able to explain this preference on the basis of either steric or electrostatic repulsions.

Snyder (43) also presented a "zeroth-order" approximation to the problem of rotational isomerism in acyclic systems. Thus, for the system XIX



XIXa XIXb XIXc

in general,

$$J_{aH} = n_a J_t + n_b J_g + n_c J_g \qquad (5)$$

$$J_{bH} = n_a J_g + n_b J_t + n_c J_g \qquad (6)$$

where the n's are mole fractions, and J_t and J_g are the <u>vicinal</u> coupling constants between <u>trans</u> and <u>gauche</u> hydrogens, respectively and J_{aH} and J_{bH} are the average <u>vicinal</u> coupling constants of hydrogens a and b. Introducing the following assumptions: $J = J_0\cos^2\phi$ describes the angular (ϕ) dependence of <u>vicinal</u> coupling constants (J); the equilibrium dihedral angle between <u>gauche</u> hydrogen is 65°; the mole fraction of conformer XIXc is zero, equations 7 and 8 can be derived,

$$J_{aH} = J_0(n_a\cos^2 185^\circ + n_b\cos^2 65^\circ) \qquad (7)$$

$$J_{bH} = J_0(n_a\cos^2 65^\circ + n_b\cos^2 185^\circ) \qquad (8)$$

and then by division of 7 by 8 and rearrangement,

$$\frac{n_a}{n_b} = K = \frac{r - 0.180}{1 - 0.180r} \qquad (9)$$

where $r = J_{aH}/J_{bH}$.

The sum of J_{aH} and J_{bH} from equations 5 and 6 is

$$J_+ = J_{aH} \; J_{bH} = (n_a + n_b)(J_g + J_t) + 2n_c J_g. \qquad (10)$$

If this equation is valid, the J_+ should be independent of changes in conformer population when $n_c = 0$. However, it is not true that invariance of J_+ means that $n_c = 0$. This type of model (equation 9) can also be transformed so as to be used for systems of the type ACH_2-CH_2B.

Snyder compared some values calculated by the use of his equations with the unpublished values obtained by Whitesides by an independent method. The agreement was very good; the average difference was only seven per cent. However, in other compounds, the agreement with published results (only seven) was not nearly as good. In general, the data showed that J_+ was insensitive to solvent changes, particularly in compounds with bulky groups. In compounds with less bulky groups, J_+ showed a greater solvent dependence, consistent with the fact that n_c was probably significantly greater than zero.

The n.m.r. spectrum of Ø-$CH(CO_2Et)$-CH_2CN was particularly interesting in that the two <u>vicinal</u> coupling constants were very nearly equal in all solvents, but the chemical shift difference between the methylene protons remained large. Thus, the methylene protons are equivalent in the spin-coupling sense, but nonequivalent in the chemical shift sense. The observation that the <u>vicinal</u> coupling constants were equal does not

imply that the nonequivalence arises from an intrinsic asymmetry effect, but could be explained by $n_a = n_b$.

Snyder points out two methods which could possibly determine the origin of magnetic nonequivalence. In the series of compounds $ACH_2C\equiv C-CXYZ$, the energy difference between conformers should be negligible. If nonequivalence was observed, then it must be due to intrinsic asymmetry. The converse is, however, not valid (i.e., the experiment is only meaningful if nonequivalence is observed). Another system would be p-XC_6H_4(p-YC_6H_4)-C-(C_6H_5)CH_2Z. If X and Y are not too large, then the conformers should all have an equal population. The observation of magnetic nonequivalence of the methylene protons would favor the intrinsic asymmetry theory.

Very recently, Ruben (44) has suggested another method to attempt to estimate the magnitude of the intrinsic asymmetry effect. For a system of the type YCX_2-CABC, the observed chemical shift difference ($\Delta\nu_{total}$) between the X nuclei will be

$$\Delta\nu_{total} = \sum_{i}^{n} X_i \Delta\nu_i \qquad (11)$$

where X_i is the mole fraction of conformer i and $\Delta\nu_i$ is the chemical shift difference between the X nuclei in conformer i. In the case where all the X_i's were equal, the nonequivalence that would result would just be the intrinsic asymmetry (the author calls this intrinsic diastereomerism, $\Delta\nu_{id}$), as shown in equation 12.

$$\Delta\nu_{id} = \sum_{i}^{n} 1/n \ (\Delta\nu_i) \qquad (12)$$

Combining equations 11 and 12 yields

$$\Delta\nu_{total} = \sum_{i}^{n}(X_i - \frac{1}{n})\Delta\nu_i + \sum_{i}^{n}(\frac{1}{n})\Delta\nu_i \qquad (13)$$

or $$\Delta\nu_{total} = \Delta\nu_{cp} + \Delta\nu_{id} \qquad (14)$$

where $\Delta\nu_{cp}$ is the contribution of conformational preference and Δ_{id} is the contribution due to intrinsic diastereomerism. Thus, as the conformer populations approach equality (as would be expected at high temperature) $\Delta\nu_{cp}$ approaches zero. However, $\Delta\nu_{id}$ should not depend on conformer population. The author pointed out that the arguments that they have proposed, namely that $\Delta\nu_{id}$ is small, depend on the judgment that the chemical shifts of X_a^1, X_a^2, X_a^3 are about equal to those of X_b^1, X_b^2, X_b^3, respectively (the symbol X_a^1 refers to nucleus X_a in conformer 1). Thus, the differences $(X_a^1 - X_b^1)$, $(X_a^2 - X_b^2)$, and $(X_a^3 - X_b^3)$ are all small and consequently their average value $(\Delta\nu_{id})$ will be very close to zero. As was pointed out before,* Gutowsky (28) attempted to make an estimate of $\Delta\nu_{id}$ by using high temperature n.m.r. data. The value he obtained, 6.7 cps for CF_2Br-CFBrCl (XX), was, however, quite small and rather uncertain.

It is possible to calculate $\Delta\nu_{id}$ directly from low temperature n.m.r. data (where the individual rotamers can be observed) using equation 12. Using data published in the literature (45,46), Raben calculated values of $\Delta\nu_{id}$ for XX and for CF_2Br-CHBrCl (XXI). These values

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* See p.140 , this thesis.

are summarized in Table 18.*

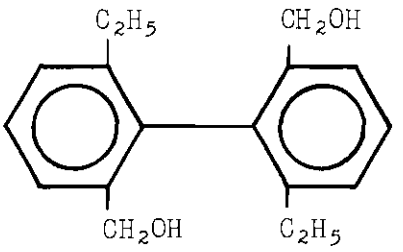
Table 18. Calculated Values of $\Delta\nu_{id}$ Compared to $\Delta\nu_{total}$.

Compound	$\Delta\nu_{id}$	$\Delta\nu_{total}$ (°C)
XX	5	95 (27°)
XXI	55	188 (30°)

Although $\Delta\nu_{id}$ was small for XX, it was quite large for XXI. Furthermore, the values of $(X_a^1 - X_b^1)$, $(X_a^2 - X_b^2)$, and $(X_a^3 - X_b^3)$ were not small; they were 155, 280, and -420 cps for XX, and 1030, 43, and -907 cps for XXI (at 56.6 Mc). Thus in cases where $\Delta\nu_{id}$ was small, the reason appeared to be because of the approximate cancellation of large terms of opposite sign. The author (44) pointed out that "great care should be taken before assuming that two nuclei which are in environments which are on first glance as similas as X_a and X_b have nearly the same chemical shift."

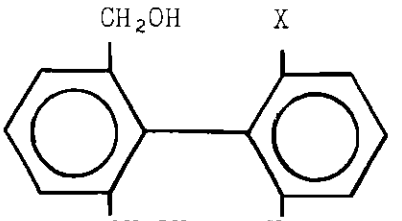
Although most of the examples of nonequivalence of geminal groups have been in compounds of the type $A\underline{B}_2C$-C-XYZ (where it sometimes has been shown that Z can equal $-C\underline{B}_2A$), other types of molecular asymmetry can induce nonequivalence. Meyer and Meyer (47) observed that the methylene protons (in the $HOCH_2$- group) of XXII were nonequivalent at room temperature. However, at about 127° a single line was observed. This was easily

* All values are in cps and refer to spectra at 56.4 Mc.



XXII

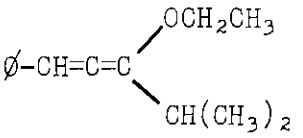
explained on the basis of hindered rotation in the substituted biphenyl. They also noted that the requirements for magnetic nonequivalence were not exactly the same as for optical activity in the biphenyl system. This, compounds of the type XXIII could show nonequivalent methylene protons, but are not capable of optical resolution.



$(X \neq Y)$

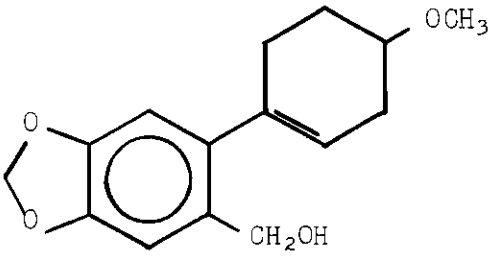
XXIII

Mislow et al. (30) noted that the methylene protons in certain doubly bridged biphenyl compounds (some of which have been resolved) were nonequivalent. Martin et al. (48) found that both the methylene protons and the isopropyl methyl groups were nonequivalent in XXIV, presumably due to the "axial" asymmetry of the alleneic system. Highet et al. (49) observed that the methylene protons of the $-CH_2OH$ group in XXV were nonequivalent under certain conditions. The n.m.r. spectrum of dilute solutions of XXV in carbon tetrachloride at room temperature



XXIV

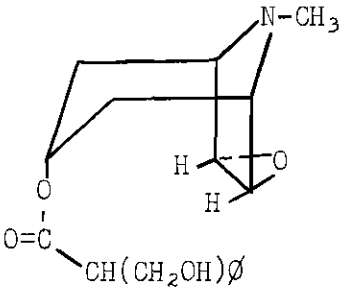
showed that the methylene protons (of the C<u>H</u>$_2$-OH group) were nonequivalent; at about 68^0 a singlet was observed. In concentrated carbon tetrachloride solution, or when dimethyl sulfoxide-d_6 was added to the dilute



XXV

solution, only a singlet was observed (for the C<u>H</u>$_2$-OH) in the n.m.r. spectrum of XXV. These observations were explained by postulating an unusually strong hydrogen bond between the -OH and the -OCH_3 groups in dilute solutions in nonpolar solvents. This would hold the methylene protons in an asymmetric environment.

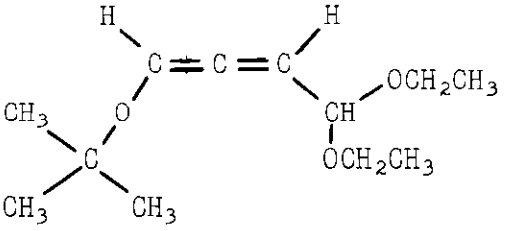
It is also possible, in some cases, for molecular asymmetry to result in the magnetic nonequivalence of <u>vicinal</u> protons. Johns and Lamberton (50) observed that the <u>vicinal</u> epoxide ring protons in the alkaloid (-)-scopolamine (XXVI) and other related compounds were nonequivalent. The authors suggested that inequalities in conformer population about the C-C bond with respect to the epoxide ring protons were responsible



XXVI

for the observed chemical shifts.

"Double" magnetic nonequivalence has also been observed in the alleneic system (XXVII) (51). In this compound the ethoxyl groups were nonequivalent, presumably due to the asymmetry of the allene system; in each ethoxyl group the methylene protons were nonequivalent (they are diasteriomeric).



XXVII

Purpose of This Research

In tri-substituted propanes of the type $X\text{-}CH_2\text{-}\overset{X'}{\underset{Y}{C}}\text{-}CH_2\text{-}X$ (where Y may be hydrogen and X' need not be the same as X) the geminal methylene protons are diasteriomeric, and may be magnetically nonequivalent. It was hoped, by studying systems of this type, that further information could be gained about the nature of magnetic nonequivalence and rotational isomerism. By varying both the nature of the X groups and the

solvents used for the n.m.r. determinations, some correlation between solvent-substituent effects and the chemical shifts and coupling constants in this system might be derived.

CHAPTER II

EXPERIMENTAL

Apparatus and Techniques

With the following exceptions and additions the apparatus and techniques in Part II are the same as in Part I.

Solutions of compounds for nuclear magnetic resonance (n.m.r.) studies were prepared gravimetrically. Percentages, where given, are therefore weight to weight. All solutions were deoxygenated by placing a very fine capillary in the n.m.r. sample tube (Varian Instruments) and bubbling dry nitrogen through the solution (ca. five minutes at room temperature for organic solvents and ca. 15 min. at 70-80° for deuterium oxide). After the solutions were deoxygenated (still under a nitrogen blanket) the n.m.r. sample tubes were sealed. Care was taken not to decompose either the solvent or compound during this operation. Generally the sealed tubes were quite symmetrical and did not cause appreciable spinning side bands.

All spectra were calibrated by the usual "side-band" technique employing a Hewlett Packard oscillator and frequency counter. On any given day, before a series of spectra were determined, the instrument was adjusted so that the 500 cps sweep width was 500 $\pm$ 1 cps, the 100 cps sweep width was 100 $\pm$ 0.2 cps, and the 50 cps sweep width was 50 $\pm$ 0.1 cps. The spectrum of any given compound was determined at least three different times on different days. It was observed that there were no

significant differences between spectra of a given compound when the sweep widths were properly adjusted.

Theoretical n.m.r. spectra were calculated, using a Burroughs B-5500 computer. The program used was written by Mr. Wayne G. Sullivan and Mr. Walter Fleming and could handle systems of seven or less protons. Reference 52 contains complete details of the calculation of theoretical n.m.r. spectra, as well as a print out of the actual program used. Approximate values for the parameters J, the spin-spin coupling constant, and $\Delta\nu$, the chemical shift difference, were obtained by trial and error and by experienced guessing. The parameters were then systematically changed (J by steps of 0.1 cps and $\Delta\nu$ by steps of 0.25 cps) until the best fit was obtained between the calculated and experimental spectra. In all cases it was assumed that the sign of the geminal coupling constant was opposite to that of the vicinal coupling constant.

Preparation and Purification of Compounds for Study

Tricarballylic Acid

trans-Aconitic acid (National Biochem Corp., 80 g.) was decolorized in boiling water using 10 g. of darco. Removal of the darco by filtration followed by evaporation of the water yielded a tan solid. This material was recrystallized from ca. 300 ml. of acetic acid and gave 50 g. of snow-white crystals, m.p. 188-189° [lit. (53) m.p. 187-188°].

trans-Aconitic acid (50 g., 0.290 mole) was hydrogenated in two 25-g. batches using a Parr apparatus. Each batch was dissolved in 200 ml. of acetic acid-water (1:1, v/v), and 5.0 g. of five per cent platinum on carbon was added. The initial hydrogen pressure was 40 p.s.i.g. After

45 min., hydrogen uptake had ceased. The mixture was filtered through a celite mat and the solids were washed with hot acetic acid-water. The filtrate was evaporated to dryness, and the resulting solid was recrystallized twice from acetic acid. This yielded a total of 35.0 g. (0.200 mole, 69%) of tricarballylic acid, m.p. 158-160° [lit. (54) m.p. 160°].

Trimethyl Tricarballylate

A solution containing 30.0 g. (0.170 mole) of tricarballylic acid, 200 ml. of anhydrous methanol, 75 ml. of 2,2-dimethoxypropane, and 3.0 ml. of concentrated sulfuric acid was boiled under reflux for three hours, and the mixture was then allowed to stand at room temperature overnight. To this mixture was added 500 ml. of water. The mixture was extracted twice with 200-ml. portions of chloroform, and the chloroform solution was dried and evaporated. Distillation of the viscous product yielded 32.2 g. (0.148 mole, 87%) of material, b.p. 110°/1.5 mm. [lit. (55) b.p. 150°/13 mm.].

Tricarballylic Acid Triamide

A mixture of 10.0 g. (0.046 mole) of trimethyl tricarballylate, 40 ml. of concentrated ammonium hydroxide solution, and 10 ml. of ethanol was stirred overnight at room temperature. The solution was then saturated with gaseous ammonia and stirred for an additional six hours. The solvents were evaporated, and the residue was recrystallized from water. This yielded 4.3 g. (0.025 mole, 54%) of a white solid, m.p. 214-216° [lit. (55) m.p. 205-207°].

β-Acetylglutaric Acid Ketodilactone

Into a 100-ml. round-bottomed flask was placed 6.82 g. (0.028 mole of dry trisodium tricarballylate [prepared from 4.96 g. (0.028 mole) of

tricarballylic acid and dried at 140°C for two hours] and 17.4 g. of acetic anhydride. The flask was equipped with a reflux condenser and was heated at 120-130° in an oil bath for a total of 24 hr. The excess acetic anhydride was distilled from the reaction mixture using first a water pump and then an oil pump. The solid was removed from the flask and was pulverized in a mortat. It was then continuously extracted with chloroform in a soxhlet apparatus overnight. The dark brown chloroform extract was dried and evaporated. The brown crystalline mass obtained was transferred to a sublimation cell and sublimed (as a melt) at 100-110° (40 μ vacuum). This yielded 2.58 g. of a light yellow crystalline solid. The solid was recrystallized once from 1:1 (v/v) chloroform-benzene and yielded 2.02 g. (0.013 mole, 46%) of glistening white needles, m.p. 99.5-100° [lit. (56) m.p. 99°]. The infrared spectrum of this compound (1.0% solution in chloroform) showed one maximum in the 5.0-6.0 μ region at 5.55 μ.

Dibenzylphenylcarbinol

A three-liter three-necked flask was equipped with a reflux condenser, a calcium chloride drying tube, a stirring motor, and an addition funnel. Into the flask was placed 700 ml. of sodium-dried ether and 6.7 g. (0.28 mole) of magnesium turnings. A small portion of a solution of 42.5 g. (0.27 mole) of bromobenzene in 300 ml. of ether was added to the flask. The reaction mixture was heated and stirred until the reaction started as evidenced by spontaneous boiling and cloudiness. The remainder of the bromobenzene was added over a one-hour period, and the solution was boiled under reflux for an additional hour. A solution of 37.9 g. (0.18 mole) of 1,3-diphenyl-2-propanone (Eastman 974-P, a thick yellow oil) in 400 ml. of ether was added to the above solution over a period of

20 min. This solution was stirred and boiled under reflux for 15 hr. Saturated aqueous ammonium chloride solution (40 ml.) was added to the reaction mixture with vigorous stirring. The solids were then filtered, and the ether layer was separated, dried, and evaporated. This yielded 48.1 g. of a thick yellow oil, which, upon standing in a refrigerator partially crystallized. The crystalline mass was placed on a Büchner funnel, and as much of the oily material as possible was removed from the crystals by suction filtration. The oily material could not be crystallized and was discarded. The product remaining on the funnel was recrystallized twice from petroleum ether (60-90°), and yielded 17.9 g. (0.062 mole, 34%) of white needles, m.p. 85-86° [lit. (57) m.p. 86-87°].

trans-1,2,3-Triphenylpropene

A solution containing 17.9 g. (0.062 mole) of dibenzylphenylcarbinol and 80.0 g. of freshly distilled acetyl chloride was boiled under reflux for four hours. The solution turned a pale yellow color. The reaction mixture was poured onto ca. 300 g. of crushed ice, and 300 ml. of ether was added. The ether layer was separated, and the aqueous layer was extracted with an additional 100 ml. of ether. The combined ether extracts were washed with aqueous potassium carbonate until the aqueous solution remained basic. The ether solution was dried and evaporated. This gave 17.0 g. of a yellow oil. The crude product was distilled and yielded 14.95 g. (0.0554 mole, 89%) of colorless oil, b.p. 168-171°/0.3 mm., [lit. (58) b.p. 220-222°/12 mm.]. This product was crystallized from petroleum ether (60-90°) after seed crystals were obtained by repeated cooling of the petroleum ether solution in a dry ice-acetone bath. Two recrystallizations from petroleum ether and one from methanol yielded

snow-white crystals, m.p. 62.5-63.5° [lit. (59) m.p. 62-63°]. The ultraviolet spectrum of the product showed λ_{max} 273 mμ, ε = 18,700 (95% ethanol).

1,2,3-Triphenylpropane

A mixture of 1.74 g. of five per cent palladium on carbon and 25 ml. of 30% ethyl acetate in 95% ethanol was equilibrated with hydrogen at room temperature and atmospheric pressure. To this mixture was added 8.71 g. (0.032 mole) of trans-1,2,3-triphenylpropene in 25 ml. of 30% ethyl acetate in 95% ethanol. After 2.5 hr., hydrogen uptake had ceased (780 ml., 95%). A small amount of di-n-butyl phthalate had, by mistake, been admitted into the reaction mixture. The solids were removed by filtration through a celite bed, and the pale yellow filtrate was dried and evaporated. An infrared spectrum (liquid film) of the oily product showed strong absorption at 5.77 μ.

The above product was mixed with a solution of 20 g. of potassium hydroxide in 150 ml. of ethylene glycol and heated at 140° for four hours. The solution was then diluted with 200 ml. of water and extracted with ether. The ether was dried and evaporated. The product was then distilled and yielded 8.59 g. of a clear, pale yellow liquid, b.p. 140°/0.10 mm. An infrared spectrum of this product (liquid film) showed weak absorption at 5.77 μ.

This material was crystallized from methanol at dry ice-acetone bath temperature; excess methanol was removed using a filter stick. Upon warming to room temperature, the product melted, and the excess methanol was removed. The product was washed twice with three-milliliter portions of methanol and was then distilled. This yielded two fractions;

No. 1, 3.28 g., b.p. 125°/50 μ, and No. 2, 1.89 g., b.p. 125°/40 μ [lit. (60) b.p. 179-181°/1 mm.]. Fraction No. 1 showed m.p. 24.0-24.8°, η_D^{30} = 1.7010 and fraction No. 2 showed m.p. 24.8-25.0°, η_D^{30} = 1.700 [lit. (60) η_D^{23} = 1.6042]. An ultraviolet spectrum of fraction No. 2 (95% ethanol) showed λ_{max} 253 (sh.), 259, 262, 265, 268 mμ, ε = 840, 1020, 970, 920, 860.

Anal.	$C_{21}H_{20}$	Calc'd:	C, 92.60; H, 7.40
	(272.4)	Found :	C, 92.64; H, 7.27

1,2,3-Trimethoxypropane

A 500-ml. three-necked flask was equipped with a reflux condenser, a stirring motor, and an addition funnel. To this flask was added 25.0 g. (0.27 mole) of glycerol and 97.8 g. (2.44 mole) of sodium hydroxide dissolved in 147 ml. of water. The contents of the flask were heated to 65° and maintained at that temperature throughout the reaction. Over a period of ca. three hours 154 g. (1.22 mole) of dimethyl sulfate was added with continuous rapid stirring. At the end of this period, the mixture was heated to 90° and stirred overnight. The reaction mixture was distilled; ca. 100 ml. of distillate was collected that showed b.p. 84-100°. Excess magnesium sulfate was added to the distillate and the solid was removed by filtration. The solid magnesium sulfate was washed with ether. The combined filtrate and washings were dried and the excess ether was removed by distillation. Distillation of the product yielded 7.0 g. (0.052 mole, 19%) of a colorless, mobile liquid, b.p. 146-147°/740 mm., η_D^{30} = 1.3959 [lit. (61) b.p. 148°/765.4 mm., η_D^{15} = 1.4069].

The above material was redistilled through a spinning-band column, and a fraction was collected, 4.0 g., b.p. 74°/46-47 mm., η_D^{29} = 1.4029.

1,2,3-Tricyanopropane Preparation

Iodoacetonitrile. Into a 1000-ml. Erlenmeyer flask was placed 106 g. (0.71 mole) of sodium iodide dissolved in ca. 600 ml. of dry acetone. The flask was covered with aluminum foil to exclude light. To this solution was added 53.1 g. (0.70 mole) of chloroacetonitrile dissolved in 100 ml. of acetone. This produced an immediate precipitate. After standing 24 hr., the mixture was filtered, and the solid was washed with acetone. The excess acetone was evaporated at reduced pressure, and the dark brown residue was distilled. This yielded 97.2 g. (0.58 mole, 83%) of a pale yellow, dense oily liquid, b.p. 64-65°/ca. 6 mm. [lit. (62) b.p. 76-77°/12 mm.]. Extreme caution was exercised in using this material since it is a very powerful skin irritant.

Ethyl α,β,β'-Tricyanoisobutyrate. In a 500-ml. round-bottomed flask 13.4 g. (0.58 mole) of clean sodium chunks were dissolved in 140 ml. of anhydrous ethanol. To this solution, 65.7 g. (0.58 mole) of freshly distilled ethyl cyanoacetate was added, and after ca. ten minutes 97.2 g. (0.58 mole) of iodoacetonitrile was added as rapidly as possible. The mixture was boiled under reflux for 0.5 hr. and was then diluted with 700 ml. of water. The aqueous solution was extracted with two 500-ml. portions of ether, the ether layer was separated, dried, and evaporated. The resulting thick black oil was distilled, yielding 29.8 g. (0.156 mole, 54%) of a thick, pale yellow liquid, b.p. 150°/1 mm. [lit. (63) b.p. 205°/18 mm.].

1,2,3-Tricyanopropane. A mixture of 2.2 g. (0.055 mole) of sodium hydroxide in 60 ml. of water (cooled to 0°) and 9.55 g. (0.050 mole) of ethyl α,β,β'-tricyanoisobutyrate was magnetically stirred until the ester

dissolved completely. The solution was then acidified to pH 1.5 with dilute sulfuric acid and boiled. A gas was rapidly evolved (carbon dioxide), and ca. one-quarter of the solution was lost because of frothing. The mixture was then allowed to cool to room temperature. When the mixture was placed in an ice bath an oil precipitated; when vigorously swirled, the oil slowly crystallized. The pale yellow crystalline material (2.54 g.) was collected and was recrystallized from ethyl acetate-chloroform. This yielded 2.30 g. (0.0193 mole, 39%) of long, colorless needles, m.p. 47.5-49° [lit. (63) m.p. 47°]. An infrared spectrum (nujol mull) of this material showed, among others, a strong band at 4.40 μ.

Dibenzylmethylcarbinol

The reaction, in anhydrous ether between 1,3-diphenyl-2-propanone (10.0 g., 0.048 mole) and methylmagnesium iodide (prepared from 13.5 g. or 0.095 mole of methyl iodide and 2.4 g. or 0.098 mole of magnesium) yielded 5.0 g. (0.022 mole, 46%) of a thick colorless oil, b.p. 140°/0.5 mm. [lit. (64) b.p. 195-200°/14 mm.].

Diethylphenylcarbinol

The reaction, in anhydrous ether between 3-pentanone (17.2 g., 0.20 mole) and phenylmagnesium bromide (prepared from 31.4 g. or 0.20 mole of bromobenzene and 5.00 g. or 0.205 mole of magnesium) yielded 16.28 g. (0.0993 mole, 50%) of a clear, colorless liquid, b.p. 71°/1.5 mm. [lit. (65) b.p. 109°/15 mm.].

Dibenzylcarbinol

A solution of 21.03 g. (0.10 mole) of 1,3-diphenyl-2-propanone in 100 ml. of absolute ethanol was slowly added over a 0.5-hr. period to a solution of 3.78 g. (0.10 mole) of sodium borohydride in 100 ml. of

absolute ethanol. After three hours, 20 g. of concentrated sulfuric acid dissolved in 150 ml. of water was slowly added to the reaction mixture. The excess ethanol was distilled from the solution and 300 ml. of water was added. The aqueous solution was extracted with two 250-ml. of portions of ether; the ether was dried and evaporated. The pale yellow residue was distilled, yielding 13.27 g. (0.0625 mole, 63%) of a clear, colorless, viscous liquid, b.p. 120-121°/0.4 mm. [lit. (66) b.p. 187-189°/12 mm.].

Citric Acid

Citric acid (Eastman 588) was dried at 78° in vacuo before use.

Sodium Citrate

This compound (Fisher Certified S-279) was dried at 78° in vacuo before use.

Triethyl Citrate

Triethyl citrate (Eastman 315) was used as received.

Trimethyl Citrate

This compound was prepared from anhydrous citric acid and dry methanol in the presence of concentrated sulfuric acid. The product was recrystallized from ethanol-water and dried at 50° in vacuo. The product showed m.p. 77.5-78° [lit. (67) m.p. 78.5-79°].

1,2,3-Trichloropropane

Trichloropropane (Eastman 5047) was distilled, and the fraction boiling at 155°/745 mm. was used [lit. (68) b.p. 154-156°/760 mm.].

1,2,3-Tribromopropane

Tribromopropane (Eastman 547) was distilled, and the fraction boiling at 94°/14 mm. was used [lit. (69) b.p. 103-104°/20 mm.].

1,2,3-Tribromo-2-methylpropane

This compound (Eastman 4231) was distilled, and the fraction boiling at 70-71°/3.5 mm. was used [lit. (69) b.p. 103°/10 mm.].

Triacetin

Triacetin (Fisher Certified A-15) was used as received.

Tribenzoin

This compound was prepared from glycerol and benzoyl chloride in pyridine solution. The product showed m.p. 71-72° [lit. (70) m.p. 71.5-72°].

CHAPTER III

DISCUSSION OF RESULTS

In this investigation, the n.m.r. spectra of a series of substituted propanes were studied. The methylene protons in these compounds were diasteriomeric, and in certain cases were magnetically nonequivalent. It was hoped that by changing the substituent groups and by studying the n.m.r. spectra of these compounds in different solvents, some insight might be gained into the nature of magnetic nonequivalence and its relationship to rotational isomerism. To this aim, a number of compounds were synthesized and their n.m.r. spectra determined.

In general, all of the synthetic reactions were quite straightforward and gave the desired products in reasonable yields. A few of these reactions will be discussed in more detail.

1,2,3-Triphenylpropene (configuration unspecified) was synthesized from dibenzylphenylcarbinol, employing the literature procedure (59). The product obtained did not appear to be a mixture of isomers (after recrystallization) as judged by its narrow melting point range. It was assigned the trans configuration on the basis of its ultraviolet spectrum, which showed λ_{max} 273 mμ, ε = 18,700 (95% ethanol). This is to be compared to the ultraviolet spectra (95% ethanol) of trans-α-methylstilbene which showed λ_{max} 272 mμ, ε = 21,000 and cis-α-methylstilbene which showed λ_{max} 267 mμ, ε = 9,340 (71).

The literature (60) preparation of 1,2,3-triphenylpropane consisted

of the reduction of dibenzylphenylcarbinol with red phosphorus and hydroiodic acid. This procedure did not seem too attractive because of the possibilities of rearrangement and cleavage reactions. The product, as prepared by this method, was reported to be a thick oil that resisted crystallization. The sample prepared by the catalytic reduction of trans-1,2,3-triphenylpropene was easily crystallized and showed m.p. 24.8-25.0°. Satisfactory analytical data were obtained.

The preparation of 1,2,3,-tricyanopropane by the hydrolysis and decarboxylation of ethyl-α,β,β'-tricyanoisobutyrate could not be carried out according to the literature procedure (63). When the ester was mixed with 50% sodium hydroxide solution a violent reaction occurred and no pure product could be isolated. When the hydrolysis was carried out in the cold, using dilute base, 1,2,3-tricyanopropane could be isolated in reasonable yield.

All of the compounds used in this investigation were purified until their physical constants (m.p., b.p., η_D) agreed with the corresponding literature values. In all cases, the n.m.r. spectra of these compounds were consistent with the structural formulas. No impurities were detected in their n.m.r. spectra.

The n.m.r. spectra that were determined during the course of this study were analyzed with the aid of a computer in the cases where ABB'CC' spectra were obtained. In cases where AB spectra were obtained, they were analyzed by the method of Pople et al. (72). As a check, the parameters calculated by the AB model were used to obtain theoretical spectra with the computer program; these parameters gave excellent agreement between the calculated and experimental spectra. It is estimated that the

coupling constants were accurate to within about ± 0.15 cps and the relachemical shifts accurate to within about ± 0.25 cps. The absolute chemical shifts (τ values) are probably accurate to within about 1 cps (approximately 0.02 ppm).

The results of these measurements are summarized in Tables 19 and 20. The data that are summarized in these tables will be discussed in three main sections: chemical shift differences, geminal coupling constants, and vicinal coupling constants.

Examination of the data revealed that in some cases there were large changes in chemical shift values in different solvents. Thus, for 1,2,3-trichloropropane the methine proton absorbed at about (solvent) 258 (neat), 254.5 (carbontetrachloride), 226.5 (benzene), 268.5 (acetone-d_6), and 265.5 cps (pyridine). The difference in the absorption position of the methine proton between benzene and acetone-d_6 solvents was 42 cps, or about 0.7 ppm. A similar effect was noted with 1,2,3-tribromopropane; the difference in absorption position between the methine proton using benzene and acetone-d_6 solvents was 57 cps, or about 1 ppm. However, with other compounds the effect was much smaller; for triacetin the maximum difference was 17.5 cps, or 0.3 ppm, and for dibenzylmethylcarbinol (for the methylene protons) the maximum difference was 18.5 cps, or about 0.3 ppm. In general, the resonance position of a given proton varied with solvent in the following order (lowest to highest field): pyridine < acetone < carbon tetrachloride and benzene. Many workers have studied the problem of solvent effects in n.m.r. spectroscopy (73). Almost all of the studies have been concerned with the effect of aromatic solvents. With nonpolar aromatic solvents such as benzene, toluene, and mesitylene

Table 19. NMR Parameters for Compounds that Give AB Spectra.

Compounds in Various Solvents	ν_A, cps*	ν_B, cps*	$\Delta\nu_{AB}$*	J_{AB}, cps
Citric acid				
Deuterium oxide	183	173	9.9	-15.9
Acetone-d_6	177.5	172.5	5.05	-15.85
Trisodium citrate				
Deuterium oxide	160	150	10.35	-15.4
Trimethyl citrate				
Deuteriochloroform	174.5	169	5.4	-15.6
Benzene	166.5	162.5	≤ 4	-15.4
Acetone-d_6	173.5	167	6.65	-15.45
Pyridine	195	189.5	5.8	-15.1
Triethyl citrate				
Carbon tetrachloride	166.5	161.5	5.25	-15.4
Benzene	171	167	4.1	-15.55
Acetone-d_6	173.5	166.5	7.35	-15.4
Pyridine	194	188	6.25	-15.0
Dibenzylmethylcarbinol				
Carbon tetrachloride	165.5	161.5	≤ 4	-13.3
Benzene	160.5	154.5	5.95	-13.35
Acetone-d_6	169.5	165.5	≤ 4	-13.5
Pyridine	179	172	7.0	-13.25
Dibenzylphenylcarbinol				
Carbon tetrachloride	190	180	10.35	-13.40
Benzene	187	180	6.95	-13.55
Acetone-d_6	195	191	4.3	-13.5
Pyridine	204	202	≈ 3	-13.4
1,2,3-Tribromo-2-methylpropane**				
Neat	236	233	3.3	-11.1
Carbon tetrachloride	236	231	5.0	-11.0
Benzene	211	202	8.9	-10.9
Acetone-d_6	241	239	≈ 2	≈ 11
Pyridine	240	238	≈ 3	≈ 11

* The values for ν_A and ν_B are in cps from internal TMS or DSS, and are given to the nearest 0.5 cps. The value of $\Delta\nu_{AB}$ is given to the 0.1 cps as determined by calculation.

** This compound showed a long range coupling of about 0.7 cps, neat and in all solvents.

Table 20. NMR Parameters for Compounds that Give ABB'CC' Spectra.

Compound in Various Solvents	ν_A cps*	ν_B cps*	ν_C cps*	$\Delta\nu_{BC}$	J_{AB} cps	J_{AC} cps	J_{BC} cps
1,2,3-Trichloropropane							
Neat	258	234	233	≈0	4.4	6.7	-11**
Carbon tetrachloride	254.5	233	232	≈0	4.4	6.7	-11**
Benzene	226.5	211	210.5	≈0	4.4	6.7	-11**
Acetone-d_6	268.5	238	237	≈0	4.3	6.5	-11**
Pyridine	265.5	235	234	≈0	4.3	6.6	-11**
1,2,3-Tribromopropane***							
Neat	267	238	332	6.3	4.6	6.7	-11.6
Carbon tetrachloride	260	235	228	7.0	4.5	6.5	-11.4
Benzene	220	203	200	≈3	4.4	6.5	-11**
Acetone-d_6	277	240	236	4.0	4.6	6.4	-11.3
Pyridine	276	238	336	≈2.5	4.8	6.5	-11**
1,2,3-Trimethoxypropane							
Neat and all solvents	too degenerate for analysis						

* The values of ν_A, ν_B, and ν_C are in cps from internal TMS or DSS, and are given to the nearest 0.5 cps. The value of $\Delta\nu_{BC}$ is given to the nearest 0.1 cps as determined by calculation.

** The spectrum was almost completely independent of this coupling constant; this value was assumed for calculation. These spectra were also independent of the order in which J_{AB} and J_{AC} were assigned.

*** This compound showed a long range coupling of about 0.5 cps, neat and in all solvents.

Table 20. Continued

Compound in Various Solvents	ν_A cps	ν_B cps	ν_C cps	$\Delta\nu_{BC}$	J_{AB} cps	J_{AC} cps	J_{BC} cps
Triacetin							
Carbon tetrachloride	308.5	253.5	244.5	9.0	4.45	5.85	-12.0
Benzene	317.5	255	246	9.3	4.4	5.8	-12.0
Acetone-d_6	313	256.5	249.5	6.8	4.4	5.8	-12.0
Pyridine	326	265	257	8.0	4.4	5.7	-12.0
Tribenzoin							
Deuteriochloroform	352	287	282	4.5	4.4	5.8	-12.0
Acetone-d_6	356.5	293	287.5	5.3	4.5	5.7	-12.05
1,2,3-Tricyanopropane							
Acetone-d_6	225	188	186	≈2	6.0	7.4	-11**
Pyridine	239.5	196.5	194.5	≈2	6.0	7.4	-11**
1,2,3-Triphenylpropane							
All solvents	too degenerate for analysis						
Tricarballylic acid							
Deuterium oxide	192	168	164	≈4	6.9	6.4	-17**
Acetone-d_6	196	171	162.5	8.3	6.8	6.3	-17.5
Trimethyl tricarballylate							
Carbon tetrachloride	188.5	162	153	8.9	6.9	6.4	-17.25
Benzene	196	162	150	12.0	6.9	6.4	-16.85
Acetone-d_6	192.5	164.5	156.5	8.0	6.9	6.4	-17.1
Pyridine	206.5	175	164.5	10.4	6.9	6.4	-16.5
Trisodium tricarballylate							
Deuterium oxide	173	148	133	14.5	8.0	7.3	-15.25

Table 20. Continued

Compound in Various Solvents	ν_A cps	ν_B cps	ν_C cps	$\Delta\nu_{BC}$	J_{AB} cps	J_{AC} cps	J_{BC} cps
Tricarballylic acid triamide							
Deuterium oxide	185.5	152.5	145	7.8	8.3	6.4	-15.55
Dibenzylcarbinol							
Carbon tetrachloride	234	161	161	≈0	5.7	6.6	-12**
Other solvents	very similar to carbon tetrachloride						
β-Acetylglutaric acid ketodilactone							
All solvents	too complicated for analysis						

it has been shown that these solvents produce a specific shielding effect on almost all protons as compared to solvents like chloroform and carbon tetrachloride (73,74). The magnitude of this effect has been correlated with the dielectric constant of the solute (74) and the acidity or electrophilic character of the solute protons (73). It has also been shown that polar aromatic solvents, such as pyridine, do not produce this specific shielding effect and in some cases may exert small deshielding effects. In general, these arguments will not explain the observed order of chemical shifts for the compounds studied in this investigation. Thus, for 1,2,3-trichloropropane, 1,2,3,-tribromopropane, 1,2,3-tribromo-2-methylpropane, and dibenzylmethylcarbinol, benzene did product a shielding effect as compared to carbon tetrachloride. However, for the other compounds that were studied in both carbon tetrachloride and benzene, the effect was either very small or in the other direction (i.e., deshielding of the protons by benzene relative to carbon tetrachloride). Clearly, some caution must be taken before accepting the theory that aromatic solvents (nonpolar) will always exert a shielding effect. In all of the compounds that were studied in both pyridine and carbon tetrachloride, pyridine solvent showed a specific deshielding effect.

The work of Anderson (75) may cast some light on the complex problem of solvent effects. He studied a series of 1,3-dioxanes and found that in general the groups at the 4, 5, and 6 positions were shielded in benzene solvent, but that groups at the 2 position were little affected or deshielded by benzene solvent (relative to carbon tetrachloride). The magnitude of these effects changed with different substituent groups. Since he thought that the 1,3-dioxanes formed a 1:1 complex with benzene,

he proposed that steric factors (which control exactly how the benzene molecule may approach the solute molecule) were important.

Thus, it seems that there is, in general, no good way to predict accurately the effects of different solvents in n.m.r. spectroscopy. Certainly any very simple considerations are apt to lead to erroneous results.

Solvent effects were also observed on the difference in chemical shifts between protons in the same molecule. Thus, the difference between the resonance position of the methine proton and the average positions of the methylene protons varied: 1,2,3-trichloropropane, 24.5 (neat), 22 (carbon tetrachloride), 15.5 (benzene), 31 (acetone), and 31 cps (pyridine); 1,2,3-tribromopropane, 32 (neat), 28.5 (carbon tetrachloride), 17 (benzene), 37 (acetone-d_6), and 38 cps (pyridine); triacetin, 59.5 (carbon tetrachloride), 57 (benzene), 60 (acetone-d_6), and 65 cps (pyridine); trimethyl tricarballylate, 31 (carbon tetrachloride), 40 (benzene), 31.5 (acetone-d_6), and 36 cps (pyridine). There did not appear to be any regular trend in these data. Generally, pyridine produced the largest differences in chemical shifts. Perhaps in these cases steric effects were important in determining exactly how the solvents molecules could interact with the nonequivalent sites within a given molecule.

The solvent was also very important in determining the extent of magnetic nonequivalence between methylene protons. Often this quantity has been related to the polarity or dielectric constant of the solvent (38,39). However, for the compounds studied here there was no completely regular trend. Thus for the compounds 1,2,3-tribromopropane,

1,2,3-tribromo-2-methylpropane, and dibenzylphenylcarbinol an inverse relation seemed to hold. For other compounds (like triethyl citrate) the opposite seemed to hold. Yet in others (like triacetin and trimethyl tricarballylate) no relationship was observed. For citric acid the observed nonequivalence was considerably higher in deuterium oxide than in acetone-d_6. However, for tricarballylic acid the opposite was true. Thus, there seems to be no general pattern to predict the effect solvent on the magnitude of magnetic nonequivalence.

Perhaps it is more logical to expect that there would not be any general pattern in the effect of different solvents on the magnitude of nonequivalence of <u>geminal</u> protons. Since these protons are diateriomeric, they are certainly in different steric environments, and would be expected to interact differently (with solvent molecules) in each compound.

The magnetic nonequivalence between methylene protons was usually largest in compounds that had the magnetically anisotropic groups ($-O-\overset{O}{\overset{\|}{C}}CH_3$, $-\overset{O}{\overset{\|}{C}}-$). However, for 1,2,3-triphenylpropane the spectra obtained were too degenerate for analysis, indicating little, if any, nonequivalence. This small effect of phenyl was also noted by Snyder (41). For 1,2,3-tricyanopropane the magnitude of nonequivalence was also very small. The spectra of both 1,2,3-triphenylpropane and 1,2,3-tricyanopropane at 100 Mc were not significantly different, which again showed the small magnitude of nonequivalence. The methylene protons of both 1,2,3-trichloropropane and 1,2,3-trimethoxypropane showed little, if any, nonequivalence at 60 or 100 Mc. Both 1,2,3-tribromopropane and 1,2,3-tribromo-2-methylpropane showed observable nonequivalence in some solvents. This might indicate

that the size of the substituent atoms was important in determining nonequivalence. Almost all of the compounds that had carbonyl groups ($-CO_1H$, $-CO_2Na$, $-CONH_2$, $-CO_2R$) showed considerable magnetic nonequivalence in all solvents. Why the carbonyl group appeared to be so effective in causing nonequivalence was not clear. Roberts et al., (38) have also noticed the large effects that a carbonyl group may have. They interpreted this in terms of a preferred conformation in which one of the methylene protons was more strongly deshielded than the other one. The only other compounds that showed considerable nonequivalence of the methylene protons were dibenzylmethylcarbinol and dibenzylphenylcarbinol. However, the closely related compounds dibenzylcarbinol and triphenylpropane showed no magnetic nonequivalence.

The chemical shift data seem to offer more support for the intrinsic asymmetry theory than the conformational preference theory. Thus, different solvents can have a large effect not only on the absolute chemical shifts of protons within a given molecule, but also on the differeces in chemical shifts. It thus seems more reasonable to interpret the changes in the magnitude of the magnetic nonequivalence of methylene protons with the inherent properties of the solvent (those that affect all chemical shifts) than with changes in conformer population caused by the different solvents. As Snyder (41) has suggested, there appeared to be little reason to correlate chemical shift differences with conformer distribution.

Only very small changes with solvent were observed in the geminal coupling constant of a given compound. Many of the differences observed were within experimental error. However, pyridine usually produced the largest changes; often the geminal coupling constant had its smallest

absolute value in this solvent. This was noticed particularly with compounds having a directly bonded carbonyl group. This perhaps indicated some specific interaction of pyridine and carbonyl groups.

The magnitudes of the geminal coupling constants were in general agreement with those reported in the literature. The large (absolute) values of these constants, in compounds containing a directly bonded carbonyl group were in accord with the "carbonyl rule" (76).

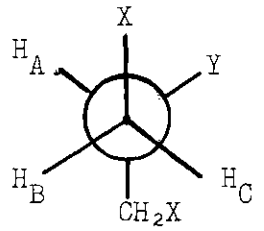
In all of the calculations it was assumed that the sign of J_{gem} was opposite of J_{vic} and was negative, as is normally believed (41). No attempt was made to find alternate parameters in which J_{gem} and J_{vic} had the same signs.

The vicinal coupling constants showed little, if any, solvent dependence, which is in substantial agreement with the work of Snyder (41). Since it is generally thought that the magnitude of vicinal coupling constants are closely related to the dihedral angle between the coupled protons, this result would indicate that there were no large changes in conformer population with solvent.

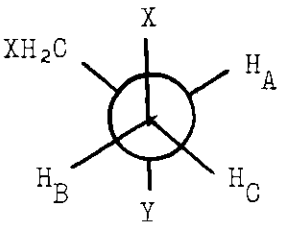
The magnitudes of the average vicinal coupling constants were inversely related to the electronegativity of the substituent groups, as is generally recognized (77). This effect is summarized below.

The compounds shown in Table 20 are analogous to the compounds studied by Snyder (41) in that the same information can be derived as in the three-spin systems. From this viewpoint, the three possible staggered conformations are shown below. Equation (9), as derived by

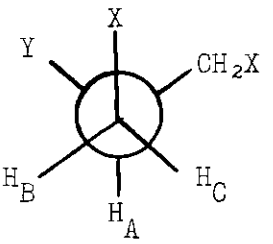
Substituent Group	Average J_{vic}
$-OCOCH_3$	5.1
$-OCO\emptyset$	5.1
$-Cl$	5.5
$-Br$	5.55
$-CO_2H$	6.6
$-CN$	6.6
$-CO_2CH_3$	6.65
$-CONH_2$	7.35
$-CO_2Na$	7.65



XXVIIIa



XXVIIIb



XXVIIIc

Snyder (43) would thus be applicable to these systems.

$$K = \frac{r - 0.180}{1 - 0.180\ r} \quad * \qquad (9)$$

This equation will allow the prediction of the ratio of the amounts of conformers XXVIIIa and XXVIIIb (conformer XXVIIIc is assumed to have zero concentration). However, it is not possible to tell which one of the two conformers is actually in the higher concentration unless it can

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* For the explanation of these symbols, see p. 151, this thesis.

be independently shown which proton (H_B or H_C) absorbs at higher (or lower) field. In general, this is not possible without the use of specific deuterium labeling (42). The results of the application of equation (9) to those data summarized in Table 20 are shown below. Only the average values (in all solvents tested) are presented since the variations in $J_{\underline{vic}}$ with solvent were within experimental error. If equation (9) is

Compound	r	K
1,2,3-Trichloropropane	1.525	1.86
1,2,3-Tribromopropane	1.392	1.62
Triacetin	1.315	1.49
Tribenzoin	1.291	1.45
1,2,3,-Tricyanopropane	1.233	1.36
Tricarballylic acid	1.078	1.11
Trimethyl tricarballylate	1.078	1.11
Trisodium tricarballylate	1.097	1.14
Tricarballylic acid triamide	1.297	1.48
Dibenzylphenylcarbinol	1.158	1.24

accurate, these results show that in none of the compounds studied was there a strong preference of either of the conformations analogous to XXVIIIa or XXVIIIb. This result was quite surprising in that it might be expected that in cases where X and Y were large groups (repulsive force between them) then conformation XXVIIIb would be strongly favored. If X and Y could exert some attractive force then conformation XXVIIIa might be strongly favored. In particular, it was surprising that 1,2,3-trichloropropane and 1,2,3-tribromopropane were so similar. For the

pair of compounds CH_2Cl-$CHClCO_2H$ and CH_2Br-$CHBrCO_2H$ (43) the average values of K (in several different solvents) were 1.90 and 4.27, respectively. For the compounds CH_2Cl-$CHCl\emptyset$ and CH_2Br-$CHBr\emptyset$, K was 1.34 and 2.68, respectively (43). In compounds like tricarballylic acid, where hydrogen bonding might produce an attractive force, little, if any, conformational preference seemed to be present. In the other compounds, such as triacetin, 1,2,3-tricyanopropane, and trimethyl tricarballylate the value of K was smaller and seemed to be more reasonable, probably reflecting the smaller size of the substituent groups.

The results for 1,2,3-tribromopropane, and possibly tricarballylic acid did not seem to agree with Snyder's work. He thought that probably steric effects were the most important factor in determining conformer preference. Thus, for these compounds, either the model presented by Snyder is not applicable, or some other factors which influence conformational preferences must be responsible. The possible nature of these other factors was not clear.

There did not appear to be any correlation between K and the chemical shift difference between methylene protons ($\Delta\nu_{BC}$). Thus if conformational preference is largely responsible for magnetic nonequivalence, it would not be unreasonable to find some correlation between K and $\Delta\nu_{BC}$. The lack of any such correlation tends to support the intrinsic asymmetry theory.

CHAPTER IV

CONCLUSIONS

Large changes with solvent were observed in the chemical shift values and the differences in chemical shifts for protons within a given molecule. These changes did not seem to be related to such properties of the solvent as dielectric constant or polarity. Benzene solvent did not exert a specific shielding effect as is usually observed. The lack of an observable solvent dependency of the vicinal coupling constant was interpreted in terms of small or negligible changes in conformer population with solvent. It thus appeared as though chemical shift data were not a reliable measure of conformer population, in agreement with the work of Snyder (41).

The equation developed by Snyder (43) that relates conformer population with the vicinal coupling constants was used to calculate values of K, the ratio of conformer populations. In particular, the values obtained for 1,2,3-tribromopropane, and possibly tricarballylic acid did not seem reasonable. No relationship between K and the chemical shift differences between geminal protons was observed, perhaps indicating that the populations of the various conformers were not important in determining the extent of magnetic nonequivalence of these protons.

In general, these data seemed to offer more support for the "intrinsic asymmetry theory," but did not exclude the possibility that the "conformational preference theory" was the most important effect.

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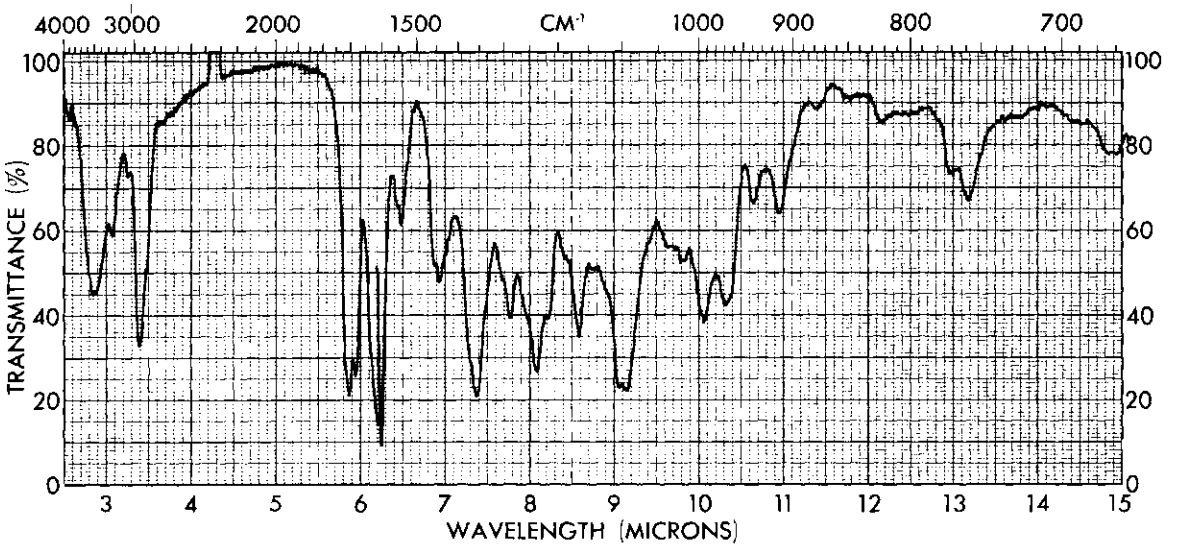

Figure 1. Infrared Spectrum of Flavensomycin.

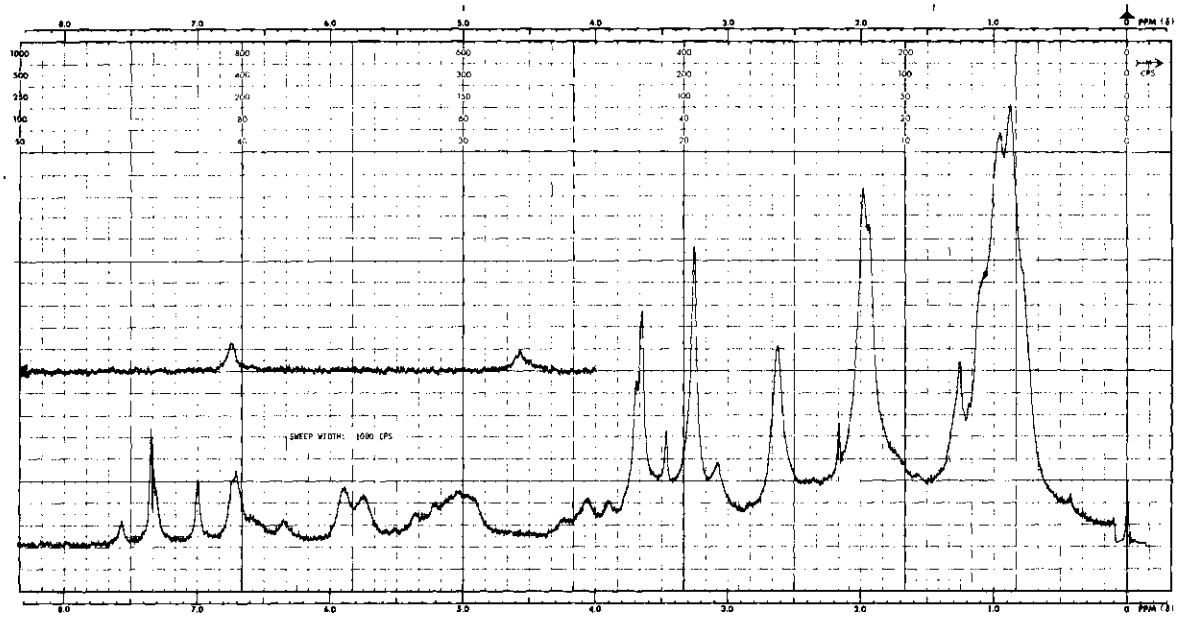

Figure 2. N.m.r. Spectrum of Flavensomycin.

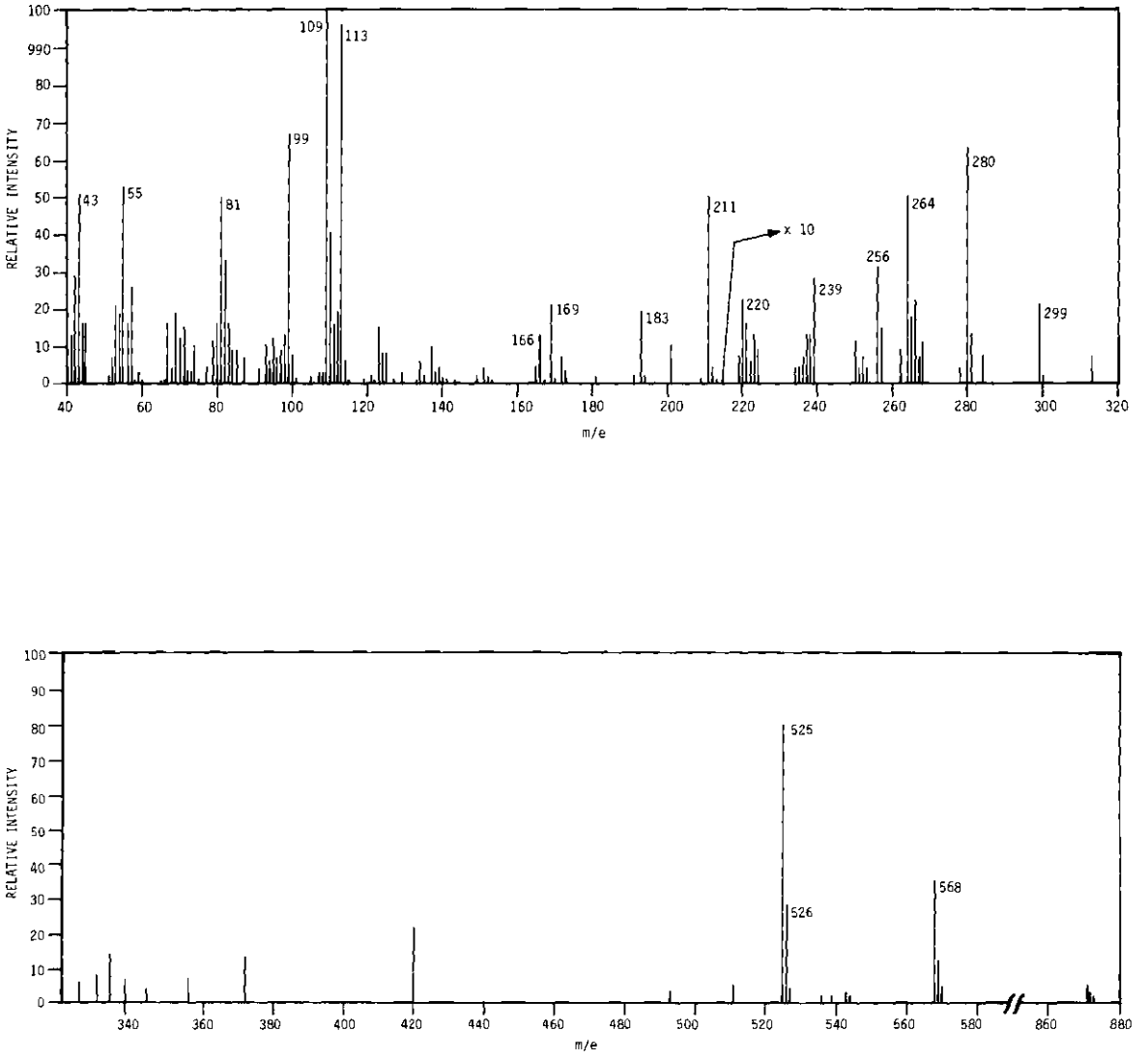

Figure 3. Mass Spectrum of Flavensomucin.

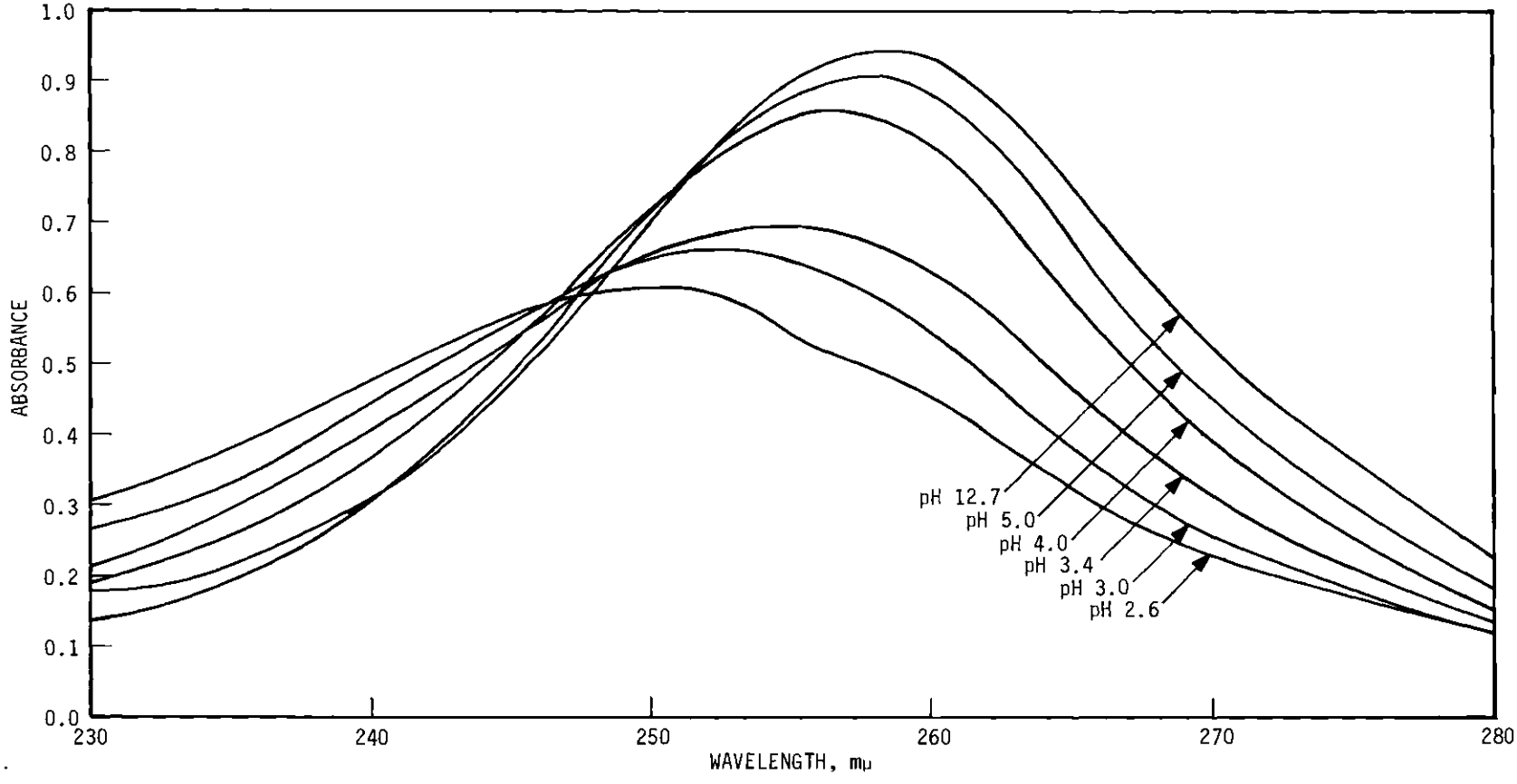

Figure 4. Ultraviolet Spectra of Dihydroflavensomycinoic Acid as a Function of pH.

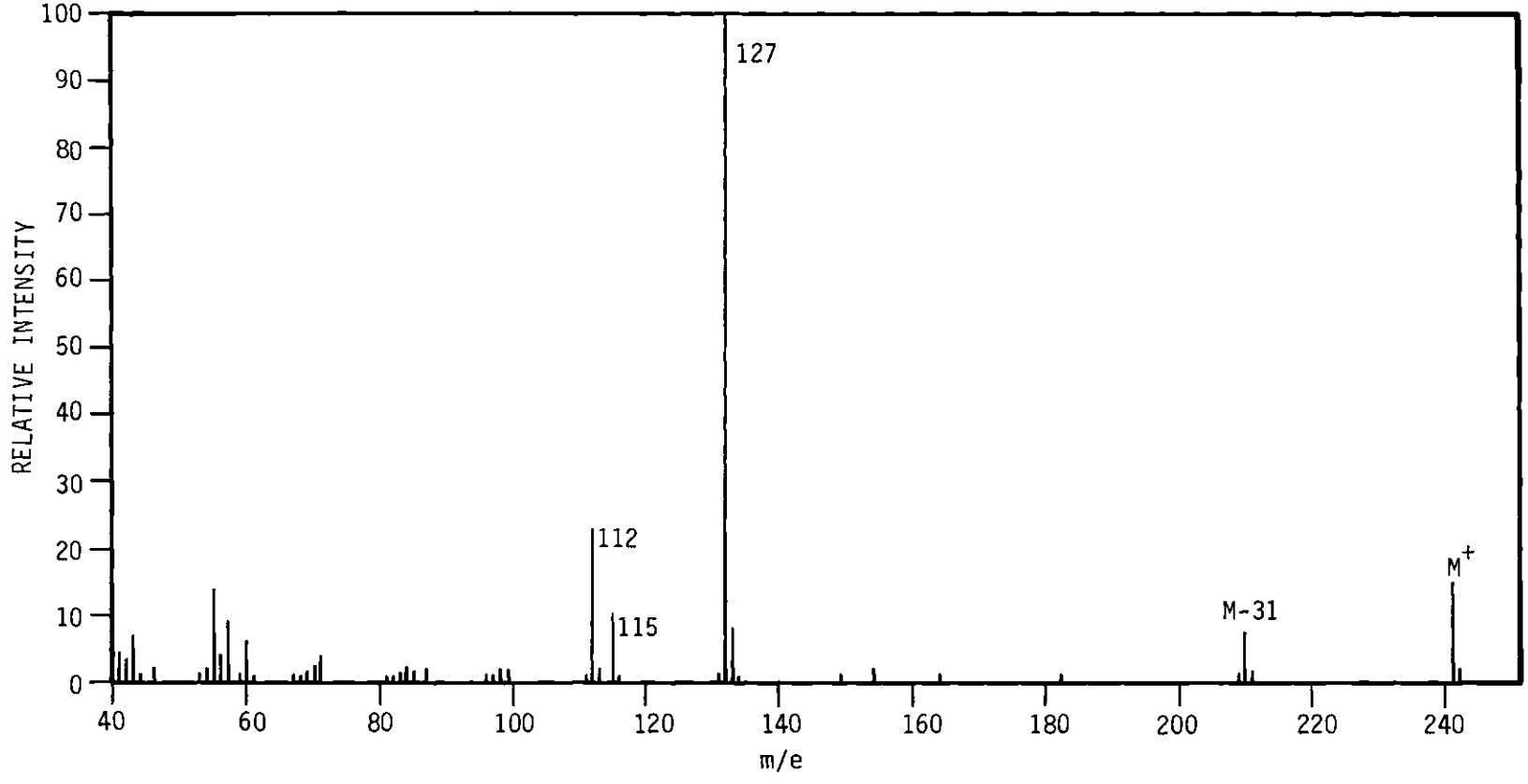

Figure 5. Mass Spectrum of Dimethyl Dihydroflavensomycinoate.

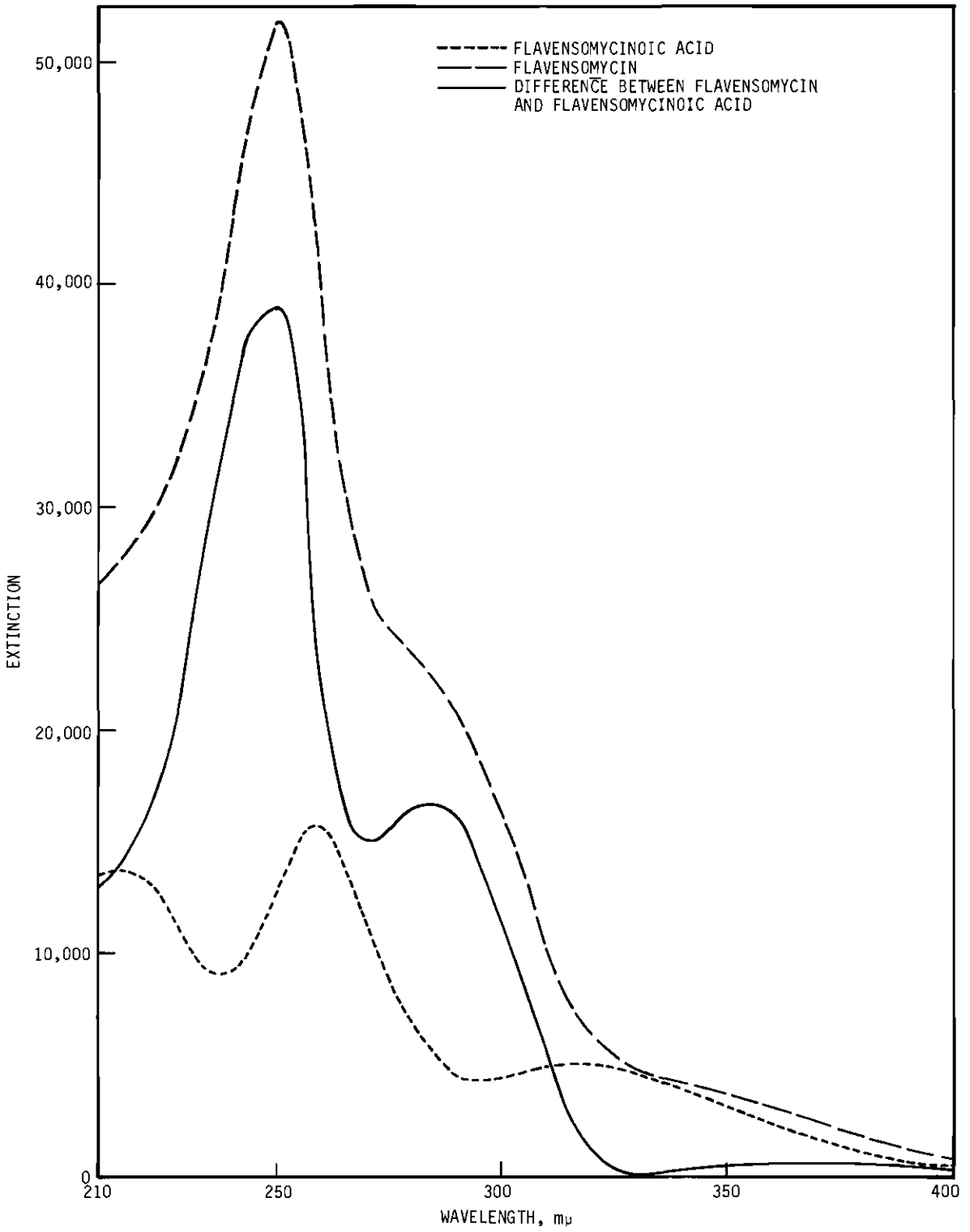

Figure 6. Difference in Ultraviolet Absorption Between Flavensomycin and Flavensomycinoic Acid.

VITA

The author was born April 23, 1940, in Los Angeles, California. He attended elementary and high schools there until 1956. At that time he and his family moved to Roslyn, New York, where his high school education was completed. He entered the Georgia Institute of Technology in September of 1958, and graduated with a Bachelor of Science degree in Chemistry in June, 1962. Graduate work at the Georgia Institute of Technology was begun in September, 1962, and in September, 1963 he was awarded a National Institutes of Health, Public Health Service, Predoctoral Fellowship.

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